



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

**AS COMUNIDADES SAVÂNICAS DO CERRADO SÃO RESILIENTES AO
FOGO?**

MARIANA CORREA DAIREL



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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.

Orientadora: Profa. Assoc. Alessandra Tomaselli Fidelis

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TÍTULO DA TESE: AS COMUNIDADES SAVÂNICAS DO CERRADO SÃO RESILIENTES AO FOGO?

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À amorosa memória de meu pai, José A. Dairel

E a cada indivíduo que participou desta jornada, contribuindo para meu crescimento
pessoal e acadêmico.

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*"Vivendo, se aprende, mas o que se aprende, mais, é só fazer
outras maiores perguntas".*

João Guimarães Rosa

RESUMO

O fogo é um elemento natural e recorrente em ecossistemas campestres e savânicos do Cerrado. A comunidade vegetal, por sua vez, é resiliente e apresenta atributos distintos que permitem sua persistência e regeneração diante de eventos de distúrbio, sendo assim uma vegetação adaptada ao regime de fogo. No entanto, a maioria das Unidades de Conservação adotaram políticas de exclusão de fogo, comprometendo diretamente a conservação destes ecossistemas abertos e a provisão de serviços ecossistêmicos. Alterações no regime de fogo, consequentemente, implicarão em modificações na estrutura e diversidade do ecossistema. Nessa perspectiva, foram implementados experimentos em diferentes áreas de fisionomias campestres e savânicas do Cerrado distribuídas por diferentes regiões do Brasil. Em cada área, foram estabelecidas parcelas (1m², com distintos delineamentos dependendo da pergunta) com intuito de avaliar o efeito da exclusão do fogo versus queimas frequentes sobre a dinâmica da vegetação e montagem da comunidade. Além disso, foram avaliados os efeitos da reintrodução do fogo na regeneração da comunidade vegetal bem como na fonte de regeneração recrutada, em áreas com diferentes regimes de queima, que haviam sido excluídas do fogo por longos períodos e que foram, posteriormente, queimadas. Com esse trabalho, esperamos contribuir significativamente para o desenvolvimento de fundamentos teóricos e práticos que se destinem a conservação, restauração e manejo de fisionomias abertas do Cerrado.

Palavra-chave: estratégia de regeneração, montagem de comunidades, fogo, Cerrado, restauração ecológica

ABSTRACT

The fire is a natural and recurrent ecological factor in open ecosystems of the Cerrado. Plant communities are resilient to fire and exhibit different traits that enable their persistence and regeneration post-fire environments, showing to be fire-adapted. However, the majority of protected areas in Brazil adopted fire-zero policies, directly affecting the conservation of open ecosystems and the provision of ecosystem services. Alterations in the fire regime will consequently result in modifications in the plant community structure, and diversity. Therefore, experiments have been implemented in different areas of open savannas of the Cerrado, distributed across different regions of Brazil. In each area, plots (1m², with different experimental design depending on the question) were established to evaluate the effect of fire exclusion versus frequent fire events on plant community dynamics and assembly. Additionally, we assessed the effects of fire reintroduction in both plant community regeneration and regeneration sources, in areas with different fire regimes which had been excluded from fire for long periods and were subsequently burned. We believe to significantly contribute to the development of theoretical and practical elements for conservation, restoration, and management of open ecosystems of the Cerrado.

Keywords: regeneration strategies, community assembly, fire, Cerrado, restoration ecology

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INTRODUÇÃO GERAL

1. O CERRADO E O FOGO

A dinâmica das comunidades vegetais ao redor do globo é frequentemente determinada por uma interação complexa entre fatores climáticos e condições do solo (Polis, 1999). Em escalas regionais, os fatores ambientais como solo, fogo e precipitação são filtros determinantes para distribuição e estrutura de ecossistemas campestres e savânicos (Bond et al. 2005; Strömberg 2011), influenciando a disponibilidade hídrica e recursos no solo, o regime de distúrbio do sistema e a produtividade primária (Grace et al. 2006; Stevens et al. 2022)

Ecossistemas campestres e savânicos são mantidos e moldados pela interação entre distúrbios, como o fogo (Bond & Keeley, 2005), e condições ambientais como sazonalidade (Strömberg 2011; Veldman et al. 2015; Buisson et al. 2019). Embora a sazonalidade não seja um fator preditor significativo nas taxas de alteração de ecossistemas abertos nas savanas tropicais (Stevens et al., 2017), esses ambientes dependem de distúrbios frequentes intrínsecos ao sistema (Buisson et al., 2019), que influenciam e mantêm sua comunidade vegetal e garantem a conservação do ecossistema.

O fogo desempenha um papel evolutivo importante na distribuição dos ecossistemas terrestres (Bond & Keeley, 2005; Bowman et al., 2009), sendo um fator estruturador fundamental na vegetação, consumindo e modificando vários ecossistemas ao redor do globo (Bowman et al., 2009). Em ambientes savânicos, o fogo é responsável por manter e influenciar a estrutura e diversidade do ecossistema (Bond & Keeley, 2005), promovendo espécies graminoides e herbáceas na comunidade vegetal, que por sua vez, é composta principalmente pelo estrato altamente inflamável de gramíneas C4 (Bond et al., 2005). O aparecimento de gramíneas C4 e o domínio desse grupo no estrato herbáceo coincide com o aumento da frequência do fogo no globo, datada no Mioceno (Beerling & Osborne 2006), impondo fortemente um filtro e pressão seletiva na coexistência com espécies lenhosas (Archer & Scholes, 1997; Beerling & Osborne,

2006; Dantas et al., 2016; Simon et al., 2009), sendo desta forma um importante fator em comunidades savânicas

As gramíneas, espécies que dominam o estrato herbáceo de savanas, apresentam estratégias adaptativas como crescimento rápido, rebrote e acúmulo de biomassa morta ao redor do indivíduo, conferindo-lhes vantagens competitivas sobre o estrato arbustivo (Bond et al., 2005). Tais atributos conferem ao estrato herbáceo alta resiliência, alimentando o ciclo do fogo (D'Antonio & Vitousek, 1992), o que garante a manutenção de ecossistemas abertos típicos de savanas. Dessa maneira, a exclusão do fogo por longos períodos pode levar à transição de ecossistemas campestres e savânicos para ecossistemas dominados por lenhosas (Abreu et al., 2017; Rosan et al., 2019; Stevens et al., 2017), resultando em perda de biodiversidade, tanto da flora quanto da fauna, além de mudanças significativas nas propriedades dos ecossistemas (Abreu et al. 2017; Wieczorkowski et al. 2022). Portanto, o fogo não apenas influencia a distribuição global dos ecossistemas (Bond et al. 2005), mas determina o processo de montagem de comunidades de ecossistemas inflamáveis, resultado das interações entre fatores bióticos e ambientais (Götzenberger et al., 2012).

A interação entre fatores bióticos e abióticos, incluindo solo, precipitação, clima, disponibilidade hídrica e fogo, atua em uma escala espaço-temporal que estrutura e limita esses ecossistemas (Bond, 2008; Lehmann et al., 2014). Sendo assim, compreender como esses fatores, especialmente o fogo, moldam as respostas da vegetação é fundamental para prever a distribuição desses ecossistemas, sua funcionalidade, provisão de serviços ecossistêmicos, e a montagem das comunidades (Götzenberger et al. 2012). No entanto, políticas de exclusão do fogo e mudanças no uso da terra representam ameaças significativas para a conservação das savanas tropicais (Buisson et al., 2019; Veldman, Buisson, et al., 2015; Veldman, Overbeck, et al., 2015).

O Cerrado evoluiu na presença do fogo ao longo de milhões de anos (Simon et al., 2009). Plantas em ecossistemas inflamáveis possuem atributos adaptativos relacionados ao fogo, como

por exemplo, proteção de gemas (tanto aéreas quanto subterrâneas, Chiminazzo et al., 2021; Pausas et al., 2018), estímulo à germinação de sementes pela temperatura e fumaça (Ramos et al. 2016; Zirondi et al. 2019), estímulo à floração (Pilon et al. 2018; Zirondi et al. 2021; Fidelis et al. 2022). E assim, o processo de retroalimentação entre o fogo e a vegetação é responsável por manter a estrutura e dinâmica das comunidades campestres e savânicas do Cerrado (Coutinho 1982, Kauffmann et al. 1994), além de criar novas condições ambientais para o recrutamento de espécies.

Após o distúrbio, o processo de regeneração da comunidade vegetal depende de uma interação complexa entre fatores bióticos e abióticos, bem como das estratégias de regeneração da comunidade. No entanto, o processo de regeneração da comunidade vegetal aérea é principalmente influenciado pela disponibilidade de uma fonte viável de propágulos (Grubb, 1977; Thompson & Grime, 1979). Essa fonte de propágulos pode estar previamente estabelecida no próprio ecossistema, permitindo que a comunidade vegetal reconstitua sua biomassa aérea por meio do banco de sementes ou banco de gemas (germinação x rebrote). Alternativamente, pode ser uma fonte externa, trazida pela dispersão com a chuva de sementes (Anderson et al., 2012; Fenner & Thompson, 2005).

O processo de regeneração pós-fogo é influenciado pela disponibilidade de recursos, pela produtividade e pelo regime de queima de cada ecossistema (Bond & Keeley, 2005; Bond & Van Wilgen, 1996). Ecossistemas que experienciam eventos frequentes de queima e de baixa intensidade tendem a favorecer espécies rebrotadoras (espécies que apresentam órgãos/estruturas portadoras de gemas que permitem a produção de novos ramos), com o banco de gemas atuando como a principal fonte de propágulos (Bond & Midgley, 2003; Pausas & Keeley, 2014), como observado nos ecossistemas savânicos (Chiminazzo et al. 2021; Pilon et al. 2021; Zupo et al. 2021). Em contraste, em ambientes sujeitos a queimas de alta intensidade mas com baixa recorrência, espécies germinadoras são beneficiadas, tendo o banco de

sementes desempenhando um papel fundamental na regeneração pós-fogo da comunidade vegetal (Keeley & Zedler 1978).

Nas formações campestres e savânicas do Cerrado, que abrigam uma rica diversidade de espécies principalmente no estrato herbáceo (Coutinho 1978) aproximadamente 40% de sua cobertura original já foi convertida áreas agrosilvipastoris (Sano et al., 2010). O remanescente está sob ameaça devido a práticas inadequadas de manejo de fogo, como a política de supressão de fogo em áreas de Cerrado (Durigan & Ratter, 2016). A ausência de fogo interfere não apenas na composição e dinâmica da vegetação (Pivello, 2011) implicando na perda de espécies herbáceas (Parr et al. 2014; Abreu et al. 2017; Wieczorkowski et al. 2022) e no adensamento de árvores (Durigan & Ratter 2016, Stevens et al. 2017, Rosan et al. 2019), mas também causa uma considerável redução na provisão de serviços ecossistêmicos (Honda & Durigan 2016) afetando assim, a resiliência do sistema (Buisson et al. 2019, 2022).

O termo resiliência é usado para designar a capacidade do sistema responder a um distúrbio, resistindo e recuperando-se, no decorrer do tempo após o evento (Hodgson et al., 2015). Entender a resiliência em ecossistemas que são mantidos por distúrbios frequentes é de extrema importância para manutenção e conservação de ecossistemas campestres e savânicos do Cerrado. O fogo é um dos principais fatores ecológicos que mantêm as fisionomias abertas de savanas e florestas como estados alternativos no cerrado (Dantas et al. 2016). Uma vez que ocorre a supressão total do fogo no cerrado (distúrbios intrínsecos) o ecossistema pode assumir distintos estados de transição (savana-floresta) que são moldados principalmente pelo fogo.

Por muito tempo a política de exclusão de fogo em Unidades de Conservação do Cerrado foi uma realidade no Brasil (Schmidt et al. 2018). No entanto, atualmente, há um intenso debate em torno do uso do fogo como ferramenta de manejo, visando à conservação e restauração dessas áreas (Durigan & Ratter, 2016; Fidelis et al., 2018; Schmidt et al., 2018; Schmidt & Eloy 2020). Nesse contexto, a reintrodução do fogo em áreas onde sua presença foi suprimida por

longos períodos torna-se crucial. Muitas são as evidências quanto aos efeitos negativos da supressão do fogo no cerrado, principalmente seus efeitos da diminuição da diversidade de órgãos subterrâneos e densidade de gemas (Ferraro et al. 2020; Bombo et al. 2022) que é a principal fonte de regeneração da comunidade vegetal após a passagem do fogo (Pilon et al. 2021; Zupo et al. 2021). Contudo, o entendimento sobre a resiliência da comunidade vegetal diante do distúrbio do fogo ainda é limitado (Buisson et al. 2019) e não sabemos muito bem como os longos períodos de exclusão podem ter afetado a resiliência. Frente ao grau de vulnerabilidade dessas áreas e seu negligenciamento diante políticas públicas, os esforços nessa direção representam uma oportunidade de integrar conhecimentos teóricos e práticos para a conservação e restauração de fisionomias savânicas do Cerrado.

Considerando a relevância da ocorrência do fogo em ecossistemas savânicos tropicais, este trabalho visa compreender o impacto do fogo na resiliência do Cerrado, por meio da análise de áreas sujeitas a diferentes regimes de queima, principalmente em áreas onde o fogo foi excluído por longos períodos. Desta forma, busca-se avaliar como a presença ou ausência do distúrbio afeta a montagem de comunidades, incluindo estrutura, composição e diversidade, bem como sua capacidade de regenerar e persistir ao longo do tempo.

2. OBJETIVOS

O objetivo geral desta tese de doutorado foi investigar o efeito da exclusão do fogo, da reintrodução do fogo no sistema bem como o efeito das queimadas frequentes sobre a resiliência da comunidade vegetal em ecossistemas savânicos do Cerrado. Objetivamos compreender como o fogo afeta a resiliência do Cerrado, através da análise de áreas com diferentes históricos de fogo, assim como avaliar as estratégias de regeneração das plantas que garantam a resiliência da comunidade após eventos de queima. Nesse contexto, foram selecionadas comunidades savânicas do Cerrado com distintos históricos de fogo: áreas sem registro de queimas (excluídas do fogo há pelo menos 10 anos) e áreas que foram recentemente queimadas

e com alta frequência de fogo. A seguir, foram elencados três objetivos específicos afim de responder o objetivo geral do projeto:

- I. Compreender como a exclusão do fogo em ecossistemas savânicos de Cerrado afeta a estrutura, composição e diversidade das comunidades vegetais.
- II. Investigar o efeito do fogo em comunidades vegetais do Cerrado que apresentam distintos históricos de fogo: avaliar a regeneração pós-fogo da comunidade vegetal, assim como sua resiliência em áreas excluídas do fogo por longos períodos.
- III. Compreender como o regime de fogo afeta as estratégias de regeneração do Cerrado.

No primeiro objetivo, avaliamos o fogo como agente estruturador na vegetação e seus efeitos na montagem de comunidades. Para isso, investigamos diferentes áreas com ecossistemas savânicos (principalmente campo sujo), distribuídas em diferentes regiões dentro do domínio do Cerrado bem como no enclave de savana localizado na Amazônia. As áreas estudadas estão distribuídas em um raio de 5000 Km ao longo do Brasil e compreende sítios com distintos fatores ambientais como condições edáficas, precipitação, e altitude, porém sempre com a comparação entre áreas excluídas do fogo há pelo menos 12 anos x frequentemente queimadas. Nesse capítulo, esperamos encontrar alterações na composição de espécies herbáceas levando a redução da diversidade de plantas devido à exclusão do fogo no sistema (Abreu et al. 2017; Wieczorkowski et al. 2022). Além disso esperamos que nas áreas excluídas do fogo haja um maior acúmulo de biomassa (principalmente de biomassa morta em pé) e redução da riqueza e biomassa de espécies herbáceas (Gomes et al., 2020; Pivello et al., 2021; Rodrigues et al., 2022).

No segundo objetivo, avaliamos como a exclusão do fogo afeta a resiliência das savanas do Cerrado. Para tanto, investigamos a regeneração de comunidades savânicas com diferentes frequências de fogo nos últimos 30 anos (alta frequência: 7 queimas, sendo a última em 2011;

baixa frequência: 15 queimas, sendo a última em 2002), as quais estavam excluídas do fogo por longos períodos e que foram, posteriormente queimadas. Neste capítulo esperamos que a exclusão de fogo levará a alterações na estrutura da comunidade vegetal, bem como alterações na abundância e composição de espécies. Portanto, quando estas comunidades excluídas do fogo forem queimadas, a regeneração da comunidade será prejudicada, uma vez que a exclusão levou à perda de espécies e consequente perda de resiliência (ver objetivo I), enquanto em áreas que apresentam maior frequência de fogo, a vegetação apresentará rápida regeneração.

No terceiro objetivo, avaliamos como o regime de fogo afeta as estratégias de regeneração do Cerrado, buscando compreender quais as estratégias de regeneração (rebrote x germinação) são mais frequentes em áreas recentemente queimadas, com baixa frequência de fogo (FE1985: 1 queima desde 1985; FE2009: 3 queimas desde 1985), e que possuem diferentes tempos desde o último fogo (35 e 11 anos, respectivamente). O regime de fogo seleciona as estratégias de regeneração da comunidade vegetal (Keeley & Zedler, 1978). Esperamos que nas áreas onde a frequência do fogo é maior, a principal estratégia de regeneração será o rebrote através do banco de gemas (Clarke et al., 2013). Por outro lado, tais áreas apresentam comunidades vegetais que não têm tempo suficiente para alimentar o banco de sementes e, portanto, a regeneração da vegetação dependerá do banco de gemas e do rebrote. As áreas excluídas do fogo apresentarão perda na capacidade de regeneração via banco de gemas (Bombo et al., 2022; Fidelis et al., 2014), e portanto, a regeneração da comunidade vegetal dependerá da germinação de sementes tanto do banco de sementes, quanto da chuva de sementes de áreas vizinhas, sendo, portanto, a germinação a estratégia de regeneração dominante (Pausas & Keeley, 2014).

O terceiro objetivo foi então dividido em dois capítulos: um capítulo voltado para entender como as estratégias de regeneração podem variar de acordo com o regime de fogo das áreas estudadas e, outro capítulo com objetivo específico de avaliar o efeito da reintrodução das queimas no banco de sementes dessas áreas que estavam excluídas do fogo por um longo

período. A descrição mais detalhada de cada capítulo da tese de doutorado é encontrada na seção “Estrutura da Tese”.

3. DESCRIÇÃO GERAL DOS MÉTODOS

3.1. Áreas de estudo

No primeiro capítulo, o estudo foi desenvolvido em seis áreas de ecossistemas savânicos do Cerrado distribuídas pelo Brasil: Parque Nacional de Brasília (PNB), Parque Nacional dos Campos Amazônicos (PNCA), Parque Nacional da Chapada dos Guimarães (PNCG), Parque Nacional Serra do Cipó (PNSC), Reserva Natural Serra do Tombador (RNST) and Reserva Particular do Patrimônio Natural – Catedral do Jalapão (RPPN, Figura 1).

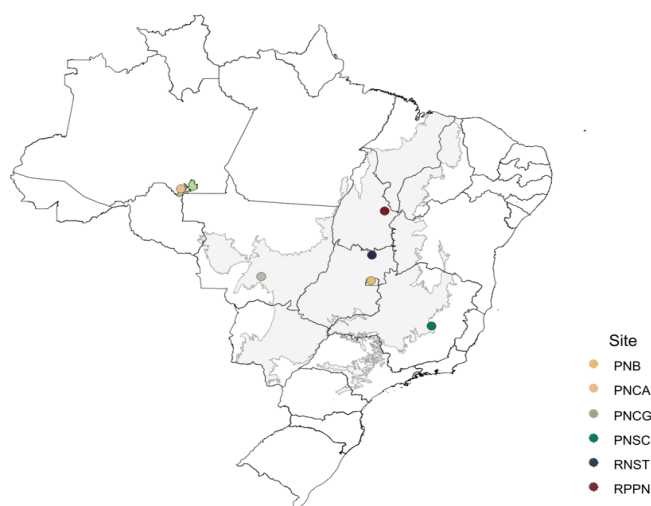


Figura 1. Distribuição das seis áreas de ecossistemas savânicos do Cerrado amostradas neste estudo: Parque Nacional de Brasília (PNB), Parque Nacional dos Campos Amazônicos (PNCA), Parque Nacional da Chapada dos Guimarães (PNCG), Parque Nacional Serra do Cipó (PNSC), Reserva Natural Serra do Tombador (RNST) and Reserva Particular do Patrimônio Natural – Catedral do Jalapão (RPPN).

Para o segundo capítulo, o estudo foi desenvolvido na Reserva Natural Serra do Tombador (RNST, 47°45'–51' W e 13°35'–38' S, 8900 ha, 560–1118 m), localizada na região

central do Brasil. Enquanto o terceiro e quarto capítulo, foi desenvolvido na Estação Ecológica de Itirapina (EEI, 47°51'-48° 00' W e 22°11'-15' S; 2.300 ha, 710-830m) localizada na região sudeste do Brasil.

De forma geral, todas as áreas de estudo apresentam ecossistemas savânicos do Cerrado, principalmente campo sujo. Nessas formações, há o domínio do estrato herbáceo-arbustivo, caracterizado por apresentar elevada riqueza de espécies (Coutinho, 1978; Eiten, 1972). O estrato herbáceo é dominado por espécies graminóides (principalmente gramíneas C4), mas também é composto por diversas herbáceas não-graminóides. Permeando a matriz herbácea, são encontradas espécies arbustivas e arbóreas que representam até 25% da cobertura e composição da paisagem (Ribeiro & Walter, 2008).

As áreas de estudo apresentam clima do tipo Cw, com verões quentes e chuvos (Outubro – Março) e invernos secos (Abril- Setembro) (Eiten, 1972). De forma geral, todas as áreas estudadas apresentam uma grande variação nas condições ambientais, incluindo a precipitação média anual (varia entre 50-1750 mm), temperatura média anual (10°C-36°C) e altitude (300-1400asl). As informações específicas de cada área de estudo estão descritas na Tabela 1.

Tabela 1. Informações gerais sobre (n=6) áreas savânicas amostradas em distintas regiões do Brasil. Dados de precipitação e temperatura foram obtidas através do WordClim 2 (Fick & Hijmans 2017).

Áreas de estudo	UF	Coordena das	Altitude (m)	Precipitação anual* (mm)	Temperatura Máx.(°C)*	Temperatura Min.(°C)*	Solo
Parque Nacional dos Campos Amazônicos (PNCA)	AM	8° 28' S 61° 38' W	220 - 224	2414	31	20	Gleissolo/ Neossolo Quartzarênico
Parque Nacional de Brasília (PNB)	GO	15°35' S 48°55' W	1115 - 1249	1573	27	16	Latossolo
Parque Nacional Chapada dos Guimarães (PNCG)	MT	15° 33' S 56° 07' W	329 - 634	1542	29	17	Latossolo
Parque Nacional Serra do Cipó (PNSC)	MG	19° 03' S 43° 42' W	1180 - 1290	1550	27	15	Litolicos/ Afloramentos rochosos
RPPN Catedral do Jalapão (RPPN)	TO	10°09' S 46°53' W	383 - 433	1750	36	10	Gleissolo/ Neossolo Quartzarênico
RPPN Serra do Tombador (RNST)	GO	13°42'S 47°52'W	770 - 831	1778	29	19	Neossolo Litolicos/ Argissolos/ Gleissolo

*valores de média

3.2. Delineamento experimental e coleta de dados

No primeiro objetivo, em cada área de estudo foram estabelecidas parcelas de acordo com os seguintes critérios: I)- distância mínima de 10m da estrada; II)- áreas com domínio de estrato herbáceo, sem influência de sombra de árvores; III)- distância mínima de 3m entre as parcelas; IV)- históricos de fogo extremos (frequentemente queimado x exclusão de fogo há pelo menos 12 anos). Para avaliação das variáveis da comunidade vegetal, foram instaladas 30 parcelas (1m x 1m, Figura 2) em cada um dos históricos de fogo. Estabelecemos um total de 360 parcelas: 30 parcelas x 2 históricos de fogo x 6 áreas estudadas.

Os parâmetros relativos à comunidade vegetal como riqueza, diversidade e estrutura foram amostrados em cada parcela de 1m² (Figura 3). A comunidade vegetal foi avaliada através da amostragem da cobertura do solo descoberto e biomassa morta (serapilheira + biomassa morta em pé), além da cobertura de todos os indivíduos de cada espécie presente na parcela através de estimativa visual (% , Figura 3). Utilizamos uma escala de cobertura que varia em categorias de 10%, exceto pelas iniciais de 1% e 5%, até o máximo de 100%. Tendo em vista, que há sobreposição dos indivíduos devido ao arranjo tridimensional das espécies na comunidade vegetal aérea, a cobertura total da comunidade dentro de cada parcela (soma de todas as espécies presentes na parcela de 1m x 1m) pode ter ultrapassado a cobertura de 100%.

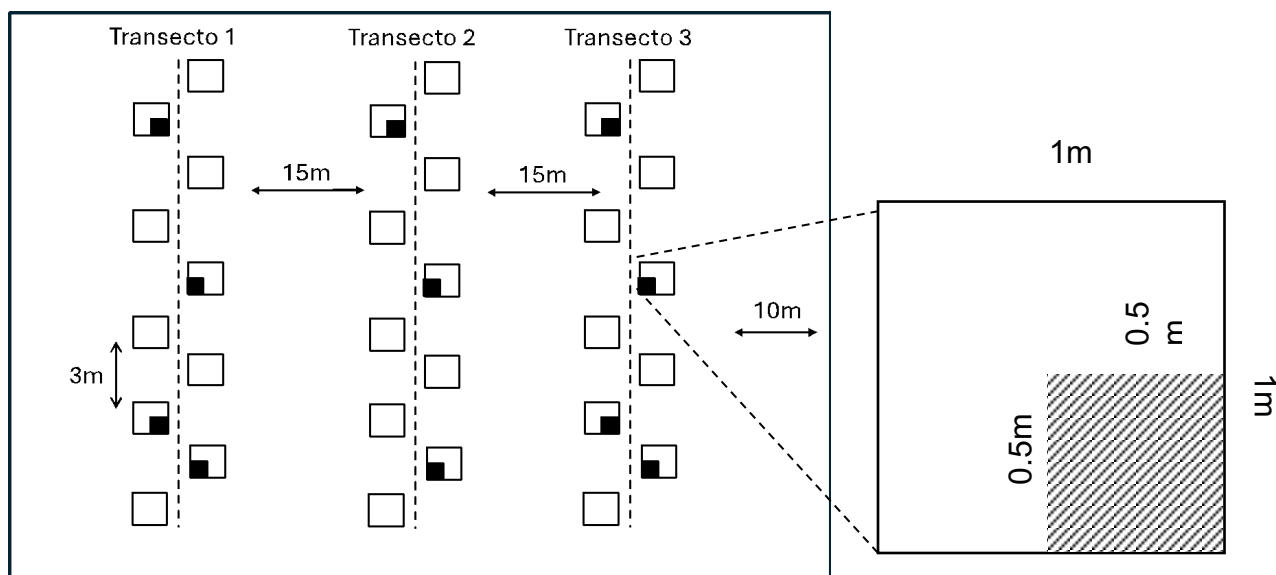


Figura 2. Esquema do delineamento experimental utilizado, seguindo os critérios propostos: distância mínima de 10 m de estrada e de árvores, parcelas estabelecidas a uma distância de 3m entre parcelas. São 10 parcelas 1x1m ao longo de 3 transectos no mesmo tamanho. A distância mínima entre transectos (linha tracejada) é de 15m. Os levantamentos da cobertura vegetal foram realizados nas parcelas 1x1m (quadrados vazios) e a coleta de biomassa para estimar a produtividade do sistema foi realizada em parcelas 0,5mx0,5m (quadrados pretos).

As espécies presentes em cada parcela foram posteriormente agrupadas de acordo com sua forma de crescimento (graminóides, herbáceas e arbustos <1,5m). Todos os levantamentos foram realizados durante as estações chuvosas (Janeiro a Março 2019 e Março 2022), quando há o pico de crescimento da vegetação.



Figura 3. Amostragem da comunidade vegetal em campo. (A) montagem dos transectos e posicionamento das parcelas 1 x 1m². (B) Imagem mais detalhada da comunidade vegetal em uma parcela de 1mx1m. (C) Estimativa da cobertura visual dos parâmetros de comunidades vegetais: solo nu, biomassa morta e cobertura por espécies vegetais.

A produtividade do sistema foi mensurada através da coleta de biomassa aérea em 10 parcelas (0.5 x 0.5m, Figura 2) por histórico de fogo. Estabelecemos um total de 120 parcelas: 10 parcelas x 2 históricos de fogo x 6 áreas estudadas. A biomassa foi coletada dentro de cada parcela (1m x 1m) utilizada para avaliar a composição da comunidade (Figura 2). A biomassa vegetal aérea foi cortada rente ao solo em cada parcela de 0.5m x 0.5m, acondicionadas em sacos de papel para serem transportadas ao laboratório (Figura 4A-C). Em laboratório, a biomassa foi triada nos seguintes componentes: biomassa morta, serapilheira, gramíneas, herbáceas e arbustos (Figura 4D). Após o processamento da biomassa, as amostras foram secas em estufa à 80°C por 48 horas e, posteriormente, pesadas.



Figura 4. Coleta da biomassa aérea. (A) Posicionando parcela de 0,5m x 0,5m para coleta de biomassa aérea. (B) Todos indivíduos que enraizam dentro da parcela são cortados rente a superfície do solo. (C) Corte da biomassa e acondicionamento em saco de papel para transporte. (D) Triagem, em laboratório, do material coletado: biomassa morta, graminóide, herbácea e arbusto.

No segundo capítulo, investigamos a resiliência da comunidade vegetal em áreas com diferentes frequência de fogo (alta frequência x baixa frequência), as quais estavam excluídas do fogo por longos períodos e que foram, posteriormente, queimadas. Para avaliar os parâmetros da comunidade vegetal de composição de espécie e diversidade, foram estabelecidas 30 parcelas fixas (1mx1m), com uma distância mínima de cinco metros, em ambas as áreas (alta frequência x baixa frequência) e tratamentos (queimadas e não queimadas; 2 áreas x 2 tratamentos x 30 parcelas, total de 120 parcelas) (Figura 5). As amostragens da comunidade

vegetal foram conduzidas da mesma forma como apresentado no delineamento do primeiro objetivo da tese.

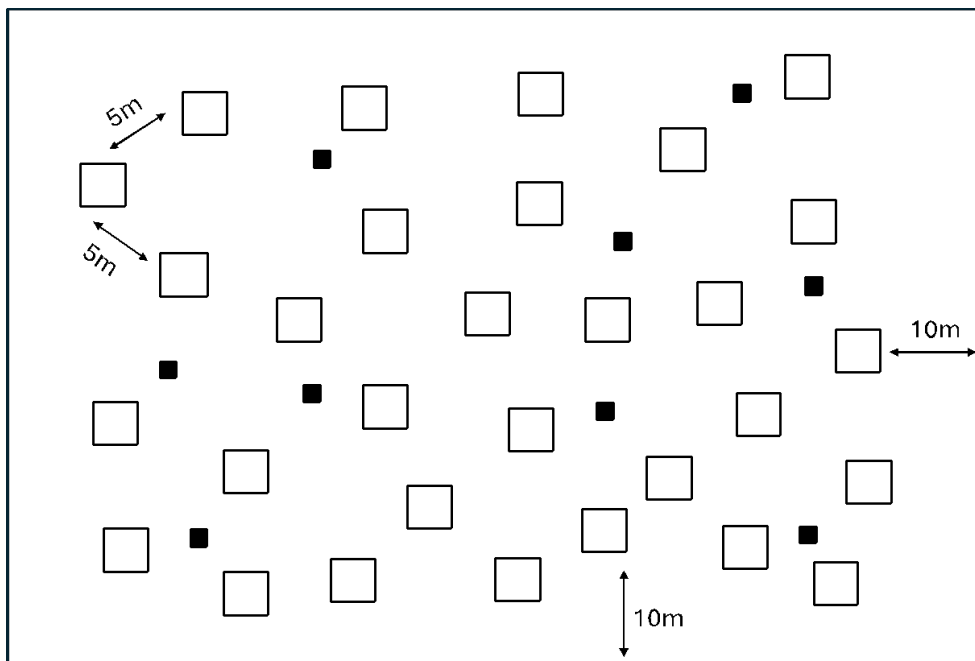


Figura 5. Esquema do delineamento experimental utilizado, seguindo os critérios propostos: distância mínima de 10 m de estrada e de árvores, parcelas estabelecidas a uma distância de 5m entre parcelas. São 30 parcelas distribuídas aleatoriamente em cada área (alta frequência e baixa frequência) e tratamento (queimadas e não queimadas). Os levantamentos da cobertura vegetal foram realizados nas parcelas 1x1m (quadrados vazios) e a coleta de biomassa para estimar a produtividade do sistema foi realizada em parcelas 0,5mx0,5m (quadrados pretos).

Além da comunidade vegetal, coletamos dados de biomassa aérea em 10 parcelas (0.5m x 0.5m) em ambas as áreas (alta frequência x baixa frequência) e tratamentos (queimadas e não queimadas). Foram coletadas um total de 40 parcelas: 10 parcelas de biomassa área x 2 áreas x 2 tratamentos. Após a coleta, os dados foram processados conforme descrito no primeiro objetivo desta tese.

No terceiro capítulo, foram selecionadas duas áreas dentro da Estação Ecológica de Itirapina (EEI) que apresentam diferentes número de eventos de queimas e distinto tempo desde a última queima desde 1985: FE1985 – excluída do fogo por 35 anos, apresentando apenas um

evento de queima em 1985 (Figura 6A-B), e FE2009 – excluída do fogo por 12 anos, apresentando três eventos de queima sendo o último ocorrido em 2009 (Conciani et al., 2021, Figura 6C-D).

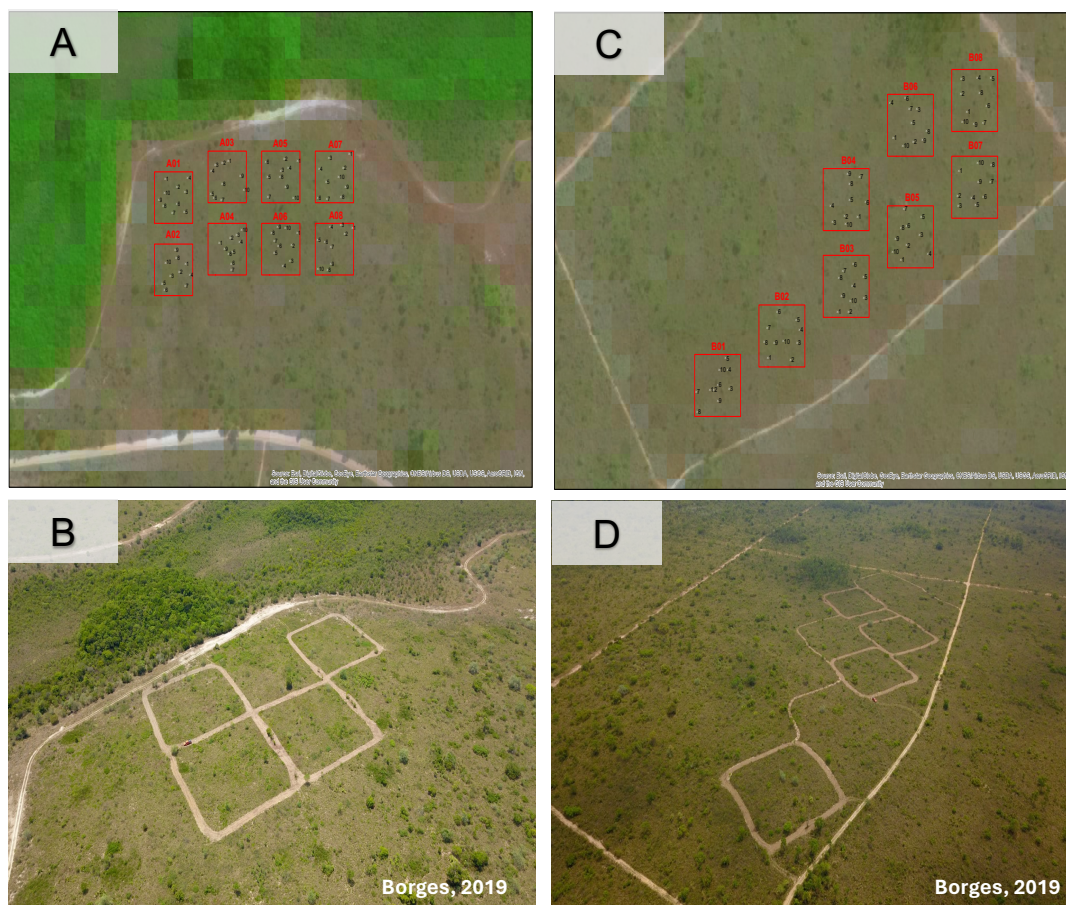


Figura 6. Implementação das parcelas fixas (50mx50m) em áreas de campo sujo na Estação Ecológica de Itirapina (EEI). (A) Área FE1985: campo sujo excluído do fogo há mais de 30 anos. Delimitação de oito parcelas experimentais. (B) Demarcação dos aceiros nas parcelas da área FE1985 que foram submetidas às queimas prescritas. (C) FE2009: campo sujo com maior frequência de queimadas dentro da EEI. Delimitação de oito parcelas experimentais. (D) Demarcação dos aceiros nas parcelas da área FE2009 que foram submetidas às queimas prescritas.

Foram estabelecidas oito parcelas fixas (50m x 50m) em cada uma das áreas (FE1985 e FE2009), das quais quatro foram submetidas às queimas prescritas e as demais parcelas não foram submetidas a nenhum tratamento (controle). A fim de garantir a independência amostral, as queimas em cada parcela foram conduzidas separadamente. Com intuito de replicar as

condições naturais do fogo no Cerrado, as queimadas prescritas foram realizadas na direção do vento (Figura 7A). As queimas prescritas foram conduzidas pela brigada de incêndio da unidade de trabalho (EEI) e acompanhadas pelo grupo de pesquisas coordenado pela professora Dra. Alessandra Fidelis (Figura 7B). Além disso, anteriormente à execução das queimadas, foi feito um aceiro de cinco metros ao redor das parcelas (Figura 6 B,D; Figura 7D).



Figura 7. Operações de queimas prescritas na Estação Ecológica de Itirapina realizadas em Agosto de 2020. (A) Linha de frente do fogo. (B) Equipe de monitoramento durante as queimas prescritas. (C) Parcela 50m x 50m imediatamente pós-queima. (D) Aceiros de 5m ao redor das parcelas submetidas às queimas prescritas.

Para avaliar como o regime de fogo afeta as estratégias de regeneração do Cerrado, foram coletados dados apenas nas quatro parcelas fixas que passaram pelas queimas prescritas.

Duas fontes de dados foram coletadas: potencial de regeneração (banco de sementes e banco de gemas) e regeneração observada (novos ramos e plântulas). Para avaliar o potencial de regeneração das comunidades, os dados foram coletados antes da reintrodução do fogo no sistema com intuito de entender a contribuição do banco de gemas (órgãos subterrâneos portadores de gemas) e do banco de sementes para regeneração da comunidade vegetal em áreas com distintos histórico de fogo (FE1985 e FE2009). Para isso, coletamos um total de 64 amostras do banco de gemas: 4 parcelas fixas experimentais x 8 amostras do banco de gemas (0.5m x 0.5m x 0.1m) x 2 áreas com distintos históricos de fogo. Já para o banco de sementes, coletamos um total de 40 amostras de solo: 4 parcelas fixas experimentais x 5 amostras de solo (0.2 x 0.2 x 0.02m) x 2 áreas com distinto histórico de fogo.

Para coletar o banco de gemas, primeiramente toda biomassa aérea foi cortada rente a superfície do solo (Figura 8A). Posteriormente, escavamos o solo com auxílio de uma pá e as amostras de solo foram peneiradas em campo (Figura 8B). Em laboratório, as amostras foram triadas a fim de separar todas as estruturas de planta presentes (Figura 8C-D). As estruturas foram classificadas de acordo com o tipo de órgão subterrâneo (como rizoma, xilopódio, raiz gemífera) e de acordo com a forma de crescimento (graminóides, herbácea, arbusto, Figura 8C-D). O número de gemas em todos os órgãos subterrâneos foi contabilizado com auxílio do estereomicroscópio.



Figura 8. Amostragem do banco de gemas em campo. (A) Coleta da biomassa área rente ao solo. (B) Amostras de solo peneiradas em campos para separação dos órgãos subterrâneos. (C-D). Triagem dos órgãos subterrâneos em laboratório para posterior contagem de número de gemas.

A coleta do banco de sementes, foi realizada em dois tempos: pré e pós-queimas (Figura 9A-B). As amostras de solo foram peneiradas em laboratório, com intuito de remover estruturas de plantas e rochas. Para avaliar o potencial do banco de sementes, as amostras de solo foram, posteriormente, transferidas para bandejas de alumínio e submetidas ao método de emergência de plântulas na casa de vegetação (Figura 9C). A coleta do banco de sementes foi utilizada para responder os objetivos do terceiro e quarto capítulo. No terceiro capítulo usamos apenas os dados do banco de sementes pré-queima. Por fim, no quarto capítulo, utilizamos os dados do

banco de sementes coletado antes da queima e após a queima, com intuito de avaliar o efeito do fogo no recrutamento do banco de sementes.

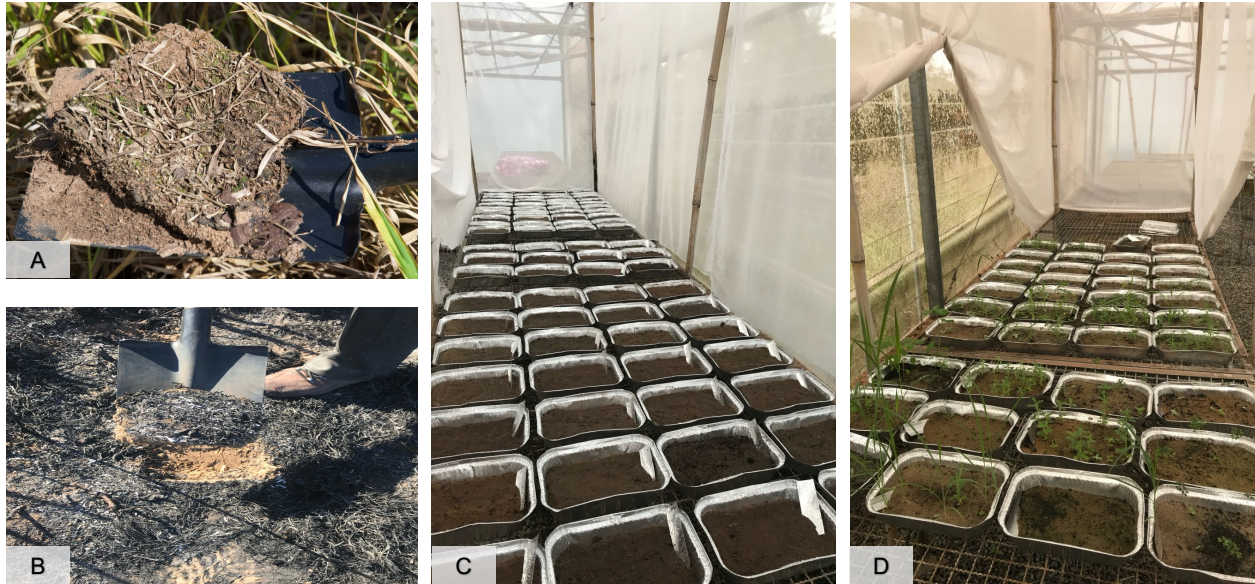


Figura 9. Coleta do banco de sementes em campo e montagem de experimentos. (A) Coleta de amostras de solo pré-queima. (B) Coleta de amostras de solo pós-queima. (C) Montagem dos experimentos de emergência de plântulas em casa de vegetação (D) Contagem das plantulâs recrutadas no banco de sementes.

Por fim, no capítulo três, para acessar a regeneração observada (ramets e plântulas) nas comunidades depois das queimas prescritas, estabelecemos um total de 60 parcelas (0.5m x 0.5m) em três das quatro parcelas experimentais: 3 parcelas fixas experimentais x 10 parcelas para contagem recrutamentos (0.5x0.5m) x 2 áreas com distintos históricos de fogo. Em cada parcela foi quantificado o número de novos ramos e o número de plântulas recrutadas após as queimas. As parcelas foram amostradas na primeira estação chuvosa após as queimas prescritas.

4. ESTRUTURA DA TESE

Esta tese está organizada em três seções: introdução geral, artigos e considerações finais. Tanto a introdução quanto as considerações finais estão redigidas em português, contextualizando a questão abordada, apresentando os resultados obtidos, bem como as implicações decorrentes do trabalho. Os artigos estão divididos de acordo com os objetivos específicos abordados, totalizando quatro capítulos, redigidos em inglês.

No primeiro capítulo, intitulado ***“Fire controls tropical savanna plant composition, diversity, and aboveground biomass independent of trees”*** (autores: Mariana Dairel, Tadeu Siqueira, Joseph Veldman, Alessandra Fidelis), selecionamos diferentes tipos de fisionomias savânicas de Cerrado no Brasil distribuídas em diversas condições ambientais, como precipitação, altitude e tipo de solo, assim como variados históricos de exposição ao fogo. Nosso objetivo foi investigar os impactos do fogo como um elemento-chave na modelagem do Cerrado em fisionomias savânicas que permaneceram por longos períodos sem queimadas, mas ainda não foram afetadas pelo processo de adensamento de árvores. Este capítulo busca elucidar como a ausência do fogo, agente estruturador no Cerrado, impacta a estrutura, a composição e a diversidade das comunidades vegetais. Esse capítulo será submetido para publicação na *Journal of Biogeography*.

No segundo capítulo, intitulado ***“Are tropical savannas resilient to fire? The example of the Cerrado”*** (autores: Mariana Dairel e Alessandra Fidelis), avaliamos a regeneração da comunidade vegetal em fisionomias savânicas do Cerrado expostas a diferentes frequências de queima (alta frequência x baixa frequência), após períodos prolongados de exclusão do fogo seguidos por eventos de queima subsequentes. as quais estavam excluídas do fogo por longos períodos e que foram, posteriormente, queimadas. Neste capítulo, para investigar a influência da exclusão do fogo na resiliência do sistema, avaliamos a estrutura, composição e diversidade da comunidade vegetal bem como a produtividade do sistema pós-queima. Este capítulo será submetido para publicação na *Journal of Environmental Management*.

No terceiro capítulo, intitulado “**How does fire regime shape regeneration strategies in tropical savannas?**” (autores: Mariana Dairel, Aline Bertolosi Bombo, Alessandra Fidelis), buscamos compreender como o regime de fogo influencia as estratégias de regeneração do Cerrado, em fisionomias savânicas com distintos históricos de exposição a eventos de queima. Para tal, analisamos a relevância das fontes de regeneração, como o banco de gemas e banco de sementes, além de avaliarmos as estratégias que foram recrutadas, como o rebrote de novos ramos e germinação de sementes, em fisionomias savânicas do Cerrado que foram reintroduzidas a eventos de queima após períodos prolongados de exclusão de fogo. períodos. Esse capítulo está sendo preparado para submissão para *Journal of Ecology*.

Por fim, no capítulo quatro, intitulado “**Fire stimulates seedling recruitment from the seed bank in the Cerrado**” (autores: Mariana Dairel e Alessandra Fidelis), buscamos compreender a importância do banco de sementes como fonte de regeneração em sistemas savânicos do Cerrado que permaneceram excluídos do fogo por longos períodos. Além disso, investigamos os efeitos da reintrodução do fogo nesses ambientes, que possuem distintos histórico de queima, sobre o recrutamento de sementes do banco de sementes. Consideramos a composição e a capacidade de recrutamento do banco de sementes antes e depois da reintrodução do fogo no sistema, visando entender o impacto do fogo nessa estratégia de regeneração da comunidade vegetal. Este capítulo foi submetido para publicação no *Journal of Vegetation Science*, já foi corrigido pelos autores e submetido novamente.

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Fire controls tropical savanna plant composition, diversity, and aboveground biomass independent of trees

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Abstract

Theory regarding the fire-dependent nature of savannas tends to focus on how fire maintains herbaceous plant communities via control of trees. In this study, we sought to determine if there are generalizable effects of fire on savanna communities that are not mediated by trees. At six locations in Brazil, spanning 5200 km and encompassing large variations in vegetation types, edaphic conditions, and climate, we sampled 6 pairs of frequently burned (F) and fire-excluded (E) tropical savannas (time since fire ranged from 15 to 19 yr), none of which had experienced forest expansion. In each location we established three transects, each with ten 1-m² plots for sampling plant community (n = 180) and ten 0.25-m² plots to measure aboveground biomass (n = 180). Ordination analyses of herbaceous species showed that frequently burned and fire-excluded savannas supported distinct plant communities. We also found that frequent fire promoted herbaceous plant diversity: local-scale species richness and within-location beta diversity were 37% and 25% greater, respectively, in frequently burned versus fire-excluded savannas. Aboveground biomass in fire-excluded savannas was twice that of those frequently burned (mean 0.44 versus 0.77 kg/m²), primarily due to the accumulation of dead biomass, most of which (90%) was grass. Although our sites lacked encroachment from trees, we did find shrub biomass was modestly (15%) higher in fire-exclude savannas. Contrary to our expectations, we found no effect of fire exclusion on forb richness or biomass. In conclusion, we show that fire exclusion can depress savanna plant diversity and community composition even in the absence of tree encroachment. Our results reinforced the idea that fire is a key ecological driver maintaining a wide variety of tropical savanna types and the efforts to conserve savannas with fire should not only focus on controlling forest encroachment.

Introduction

Grassy biomes are globally distributed across a wide range of climatic and edaphic conditions, including where its environmental factors can also support forests (Staver et al., 2011, Lehmann et al., 2014, Veldman et al., 2015). In the tropics, savannas evolved with, and are maintained by, endogenous disturbances regimes involving fire and herbivores (Bond & Keeley, 2005; Parr et al., 2014; Buisson et al., 2019). The consequences of fire suppression to savannas have received a great deal of attention once without fire, forest expansion leads to a loss of savanna biodiversity, including changes in plant composition and animal diversity (Parr et al., 2014; Abreu et al. 2017). While it is clear that fire has a major role in consuming aboveground biomass and promoting diversity in tropical savannas, it has been difficult to evaluate the effects of fire shaping plant communities independent of effects mediated by trees. Also complicating matters, fire exclusion is not the only driver of woody encroachment and losses in herbaceous diversity. Indeed, other drivers include changing precipitation (Criado et al., 2020), elevated atmospheric CO₂ (Devine et al., 2017; Stevens et al., 2017), and mismanaged livestock (Stevens et al., 2017; Wigley et al., 2010) has been widely associated with woody plant increase and its role in structuring woody cover gradient in grassy biomes.

Tropical savannas are characterized by a continuous layer of herbaceous plants that are promoted by fire (Bond & Keeley, 2005) and scattered, fire-tolerant woody species, shrubs and trees (Chiminazzo et al., 2021; Ratnam et al., 2011). The herbaceous layer is mainly composed of C₄ grasses and forbs with functional traits that enable them to survive fires and then rapidly resprout from bud banks (Parr et al., 2014; Strömberg, 2011). Rapid growth of grasses is critical to providing the fuel required for frequent fires (Linder et al., 2018; Osborne, 2008). Thus, grasses not only respond to fire, but promote fire in ways that it acts as a strong filter and selective pressure influencing the coexistence of herbaceous

and woody species (Bond, 2008; Pausas & Bond, 2019;). But what is the response of herbaceous plant communities to variation in fire regimes, between frequently burned and exclusion of fire, when there is no woody encroachment?

Current thinking about the positive effects of fire on savanna plants comes from observation of what happens when fire is excluded. Without fire, tropical savannas accumulate dead material (Buisson et al., 2019; Parr et al., 2014) which can limit light reaching the ground layer and lead to loss of several light-demanding species (Pilon, Durigan, et al., 2021). In addition, fire exclusion leads to several consequences for the ecosystem function, including declines in the belowground bud bank and storage organs (Bombo et al., 2021, 2022; Fidelis et al., 2014), which in turn reduces belowground carbon stocks (Bombo et al., 2022; Teixeira et al., 2022). Another consequence of fire exclusion is changing in herbaceous community dynamics, in which fire-dependent species fail to flower and fruit (Fidelis et al., 2022; Fidelis & Zironi, 2021; Pilon et al., 2018).

Across Brazil, tropical savannas are widespread and characterized by frequent fires (Overbeck et al., 2022) that have significant ecological and evolutionary effects on their dynamics for the past 5 to 10 millions of years (Simon et al., 2009). Occurrence of savannas under high precipitation in the Amazon is strong evidence that fire, not just climate drives the distribution of forest and savanna in Brazil (Overbeck et al., 2022). Indeed, other environmental drivers shapes our tropical savannas such as as *Campos rupestres*, where forests are edaphically constrained due to the shallow soils and rocky outcrops, fire events are common and plants are well-adapted to burning (Overbeck et al., 2022; Silveira et al., 2016). Overall, it is challenging to disentangle the role fire plays in shaping savannas, because some grassy ecosystems seem to depend on fire to avoid being overrun with trees, while others would remain open even in the absence of fire and yet burn regularly.

In this study we sought to understand the role of fire in savanna plant community assembly. To unveil the direct effects of fire, independent of trees, we studied savannas that had not experienced forest encroachment. We focused on key variables related to species composition, diversity, and aboveground biomass in pairs of frequently burned savannas (2 to 4 years since fire) and fire-excluded savannas (15 to 19 years since fire). We sampled 180 pairs of plots in six distinct savanna locations that spanned a wide geographic area (Fig. 1) and that encompassed a broad range of environmental conditions. We hypothesized that fire exclusion leads to changes in herbaceous plant composition and reduced plant diversity. We also expected that fire-excluded plant communities would have greater shrub biomass and reduced richness and biomass of forbs. Finally, we expected that fire exclusion would lead to higher accumulations of aboveground biomass compared to frequently burned savannas. We expected our results to demonstrate the importance of frequent fire management for the conservation of tropical savannas plant communities, even in places that are not yet experiencing woody encroachment.

Methods

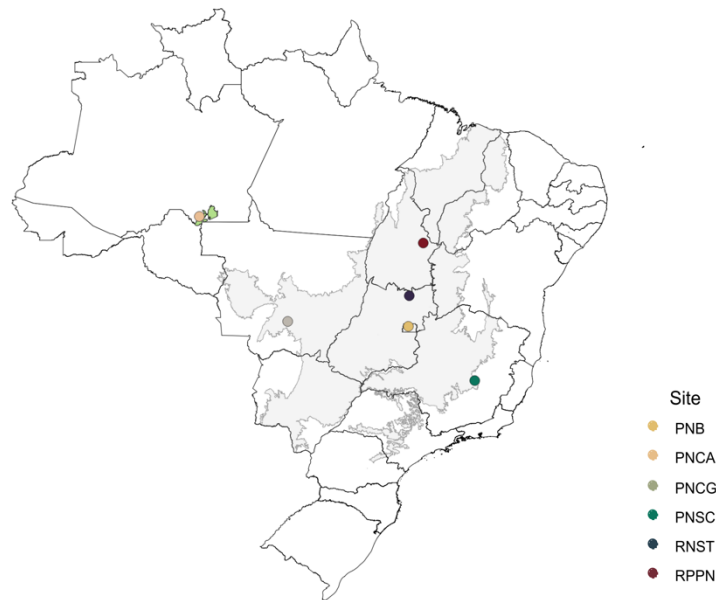
Study area

We sampled grassy ecosystems in six different locations spanning 5200 km across Brazil (Fig.1). We selected these locations to encompass variations in vegetation physiognomies (savannas and grasslands), plant species composition, edaphic conditions and climate (see Table 1).

Table 1. General information about the different sampling sites of tropical open ecosystems including type, coordinates, temperature, precipitation, altitude, fire regime (frequently burned – B, fire-excluded – E), time since last fire in relation to the sampling period (in years) and year of the last fire. Year-round average temperatures and precipitation were obtained through WordClim2 data set (Fick & Hijmans 2017). Abbreviation means: PNSC- Parque Nacional da Serra do Cipó; PNB- Parque Nacional de Brasília; PNCA- Parque Nacional dos Campos Amazônicos, PNCG- Parque Nacional da Chapada dos Guimarães; RNST- Reserva Natural Serra do Tombador and RPPN- Reserva Particular do Patrimônio Nacional Catedral do Jalapão.

Sites	Open ecosystem type	Coordinates	Temperature (min. - max. year-round average)	Precipitation (annual)	Altitude (a.s.l.)	Soil	Fire regime	Time since last fire	Year of the last fire
PNSC	Grasslands (<i>campos rupestres</i>)	-19°17'11.09"S; -43°35'43.34"W	15°C - 27°C	1550 mm	1180m	Lithosol/ rock outcrops	B	4	2014
		-19°14'50.37"S; -43°30'48.81"W			1290m		E	19	1999
PNB	Savanna	-15°40'31.61"S; -47°53'1.77"W	16°C - 27°C	1573 mm	1249m	Latosol	B	2	2018
		-15°40'30.82"S; -47°53'2.49"W			1115m		E	12	2007
PNCA	Savanna	8°28'35"S; 61°38'48"W	20°C - 31°C	2414 mm	224m	Quartz-arenite Neosol/ Latosol	B	2	2017
		8°25'59"S; 61°38'49"W			220m		E	16	2003
PNCG	Savanna	15°26'6.88"S; 55°51'37.59"W	17°C - 29°C	1542 mm	634m	Latosol	B	2	2017
		15°21'1.44"S; 55°49'50.33"W			329m		E	14	2005
RNST	Savanna	13°40'53.50"S; 47°48'37.26"W	18.5°C - 29°C	1778 mm	831m	Neosol- Lithosol/ Gleysol	B	2	2017
		13°39'30.15"S; 47°49'27.75"W			770m		E	17	2002
RPPN	Savanna	10°09'33.09"S; 46°53'51.11"W	10°C - 36°C	1750 mm	383m	Quartz-arenite Neosol/ Latosol	B	2	2020
		10°10'55.06"S; 46°54'10.01"W			433m		E	18	2004

122



123

124 **Figure 1.** Distribution of the six grassy ecosystems selected: Parque Nacional de Brasília
 125 (PNB), Parque Nacional dos Campos Amazônicos (PNCA), Parque Nacional da Chapada
 126 dos Guimarães (PNCG), Parque Nacional Serra do Cipó (PNSC), Reserva Natural Serra do
 127 Tombador (RNST) and Reserva Particular do Patrimônio Natural – Catedral do Jalapão
 128 (RPPN). Sites are displayed in different colors.

129

130 We selected six study locations in national parks and private reserves with reliable
 131 records of fire history for at least the past two decades that contained both frequently burned
 132 (B) and fire-exclude (E) sites with no sign of woody encroachment (Fig.2). Four locations
 133 are savannas of the cerrado: Parque Nacional de Brasília (PNB, Fig.2A), Parque Nacional
 134 da Chapada dos Guimarães (PNCG, Fig.2C), Reserva Natural Serra do Tombador (RNST,
 135 Fig.2D) and, Reserva Particular do Patrimônio Natural – Catedral do Jalapão (RPPN,
 136 Fig.2E). Our wettest location, Parque Nacional do Campos Amazonicos (PNCA, Fig.2B), is
 137 a savanna of the Amazon, which exists as an enclave in a region that is predominantly

forest. A sixth location was a grassland of campo rupestre in the Parque Nacional da Serra do Cipó (PNSC, Fig.2F). Although, savanna of the Amazon (PNCA) and grasslands of campos rupestres (PNSC) are considered to be edaphic-driven ecosystems due to soil depth at PNSC and water table level at PNCA thought to be the main factors that limit trees in those grassy ecosystems (Silveira et al., 2016; Overbeck et al., 2022), they are also fire-prone, typically burning every three to four years at PNCA (Alves & Pérez-Cabello, 2017), and burning around six years in PNSC (Alvarado et al., 2017).

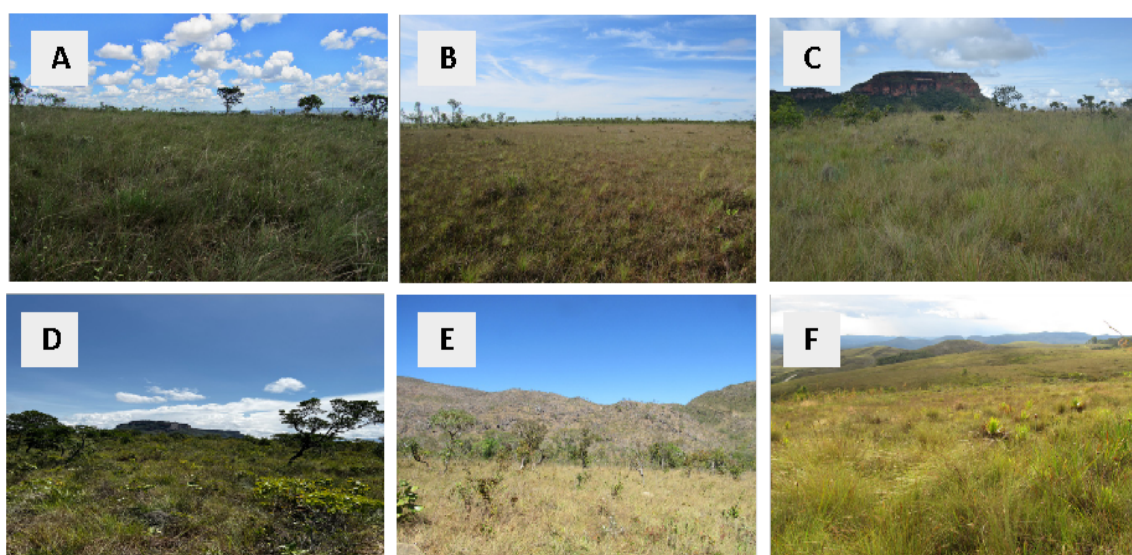


Figure 2. Study locations of grassy ecosystem distributed throughout Brazil. **A** - Parque Nacional de Brasília (PNB). **B** - Parque Nacional dos Campos Amazônicos (PNCA). **C** - Parque Nacional da Chapada dos Guimarães (PNCG). **D** - Parque Nacional Serra do Cipó (PNSC). **E** - Reserva Natural Serra do Tombador (RNST). **F** - Reserva Particular do Patrimônio Natural – Catedral do Jalapão (RPPN).

In all locations, there is a well-marked rainy season (October to March) and dry seasons (April to September; Eiten, 1972). Mean annual precipitation (MAP) among study locations ranges from 1483 to 2414 mm (Table 1). Overall, locations showed a broad range

of environmental conditions, including variations in mean annual temperature (MAT, 16°C–31°C), altitude (220 – 1290 a.s.l.), and soil types (see Table 1).

Experimental design and sampling data

We carried out vegetation surveys in all six locations during the rainy season (see Table 1). In each location we selected two sites according to the following criteria: I) grassy ecosystems with a continuous herbaceous layer, without shrub/tree shading; II) recent (past two decade) fire history that included recent burning (within past 4 years) or fire-excluded (>12 years since fire); III) - minimum distance of 10m from a road. In each site we randomly positioned three 50m transects, along which we sampled ten evenly spaced plots of (1 x 1 m). Sites ranged greatly in size a; to capture the variation within sites, transects varied in distance from one another, but were never closer than 15 m (more details see Supplementary Information – Table S1). As such, at the largest spatial scale we had 6 locations, while at the smallest spatial scale (within each location), we had 30 pairs of plots corresponding to frequent burn and fire-exclusion treatments, for a total of 360 1-m² plots sampled.

To assess vegetation structure and community composition, we visually estimated the percent cover of bare soil, dead biomass (litter and standing dead biomass), and for each herbaceous and woody species (up to 150 cm height) in each 1-m² plot. Vegetation cover was visually estimated throughout categories of 10%, except for the initial ones being 1% and 5%. To avoid observer bias, only the first author conducted all cover estimates. We identified plants to genus or species level when it was possible according to regional floras for each location, Brazilian Flora - REFLORA (Flora e Funga do Brasil) and *speciesLink* (<http://www.splink.org.br>). We also classified plants according to their growth forms as graminoids (grasses and sedges), forbs or shrubs.

To measure aboveground biomass, we randomly selected three to four 0.25m² plots within ten of the 1m² plots along each transect (total of 120 clip plots). Clipped all aboveground biomass the ground level and sorted it in to dead biomass (litter and standing dead) and live biomass, which was further sorted into growth forms. After sorting, we oven-dried samples at 80 °C for 48h before weighing.

Data analyses

To assess whether fire history influenced plant community composition, we first applied a Principal Coordinates Analysis (PCoA) on the species composition matrix that included all sites, using the *cmdscale* function in the R package *vegan* (Oksanen et al., 2019). This PCoA was based on a Bray-Curtis dissimilarity matrix. We then, ran a permutational multivariate analysis of variance (PERMANOVA) using the *adonis* function in the *vegan* package (Oksanen et al., 2019), using fire history (frequent burn or fire exclusion) as a fixed factor and location as a random factor. We ran the PERMANOVA with 10000 permutations using *strata* function to limit permutations only among fire history within locations.

To test for differences in aboveground biomass (dead and live biomass within each growth form: graminoids, forbs, and shrubs) and plant diversity in the frequently burned and fire-excluded, we fit Generalized Linear Mixed Models (GLMM), with fire history (B and E) as fixed factor and location (Table 1) as a random factor. As response variables, we used local-scale species richness and beta-diversity. Species richness was estimated with the *estimateD* function in the *iNEXT* package (Hsieh et al., 2016) and calculated as the mean number of species per 1 m² in each transect. For beta-diversity, which we calculated among the 10 plots within each transect, we used the *beta.pair* function in the *betapart* package (Baselga, 2010). For both richness and beta-diversity, we calculated the site-level mean (n=6) based on the mean from each transect, using *dplyr* package (Wickham et al., 2023).

All analyses were done with the R software® (R Core Team 2021) and for data visualization, we used *ggplot2* package (Wickham, 2016) and *wesanderson* package (Ram et al., 2023).

Results

In all six locations combined, we found a total of 468 plants that included 149 species of graminoids, 210 forbs, and 109 shrubs. PCoA axes 1 and 2 explained 16 and 10 % of the variation in species composition; with frequently burned and fire-excluded sites separated along axis 1 (Fig.3). The PERMANOVA confirmed a statistical effect of fire history on species composition ($p < 0.001$, $F = 9.626$, $R = 0.361$; Fig. 3).

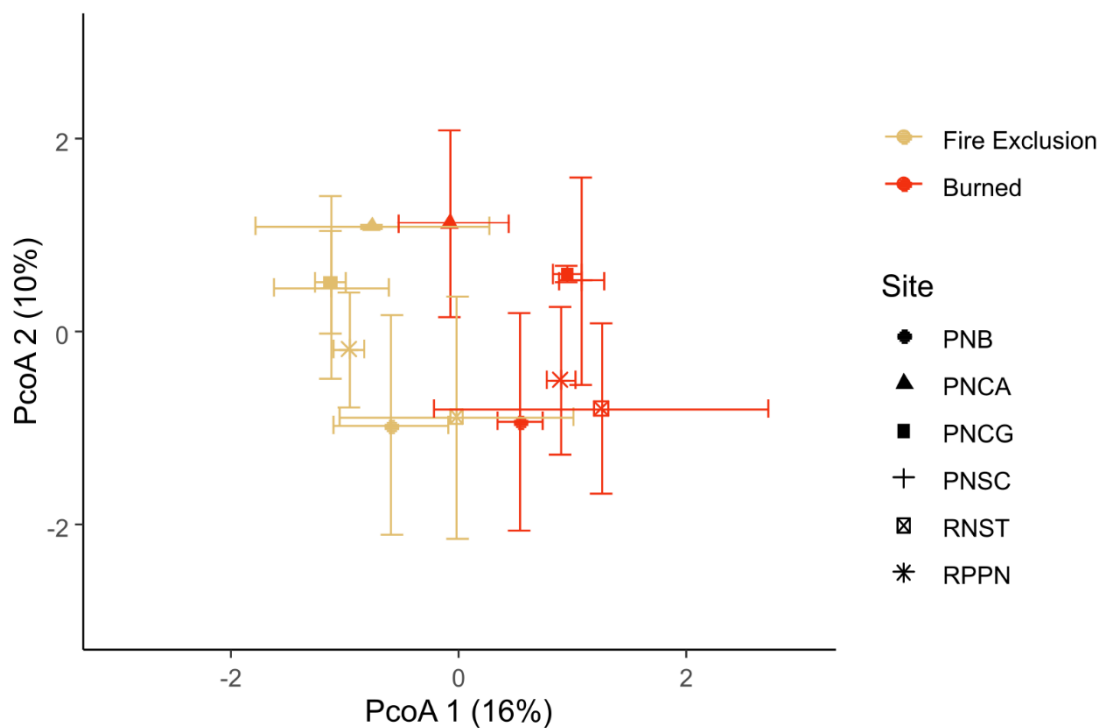


Figure 3. Principal Coordinate analysis (PCoA) of plant community composition in frequently burned and fire-excluded savannas at six location in Brazil: Parque Nacional de Brasília (PNB), Parque Nacional dos Campos Amazônicos (PNCA), Parque Nacional da Chapada dos Guimarães (PNCG), Parque Nacional Serra do Cipó (PNSC), Reserva Natural Serra do

Tombador (RNST) and Reserva Particular do Patrimônio Natural – Catedral do Jalapão (RPPN). Error bars represents our smallest spatial scale (within each location) distribution, by 30 pairs of plots corresponding to frequent burn and fire-exclusion treatments.

We found that sites that burn frequently had 37% more species compared to sites where fire is excluded (Fig.4, $p < 0.01$, $z = 2.417$, $R = 0.563$). Frequently burned sites supported higher local-scale species richness of graminoids (B, range 7-23 species in respective locations, see Table S2 – Supplementary information) then fire-excluded sites (E, range 5-20 species, see Table S2). Contrary to our expectations, fire regime did not affect local-scale richness of forbs, being similar in both treatments (B: range 7-29 species and E: range 8-28 species, see Table S2). Similarly, we found no difference in shrub richness; frequently burned and fire-excluded sites supported the same amount of local-scale richness (B: range 3-16; 1-3sp/m² and E: range 1-12; 1-3 species/m²).

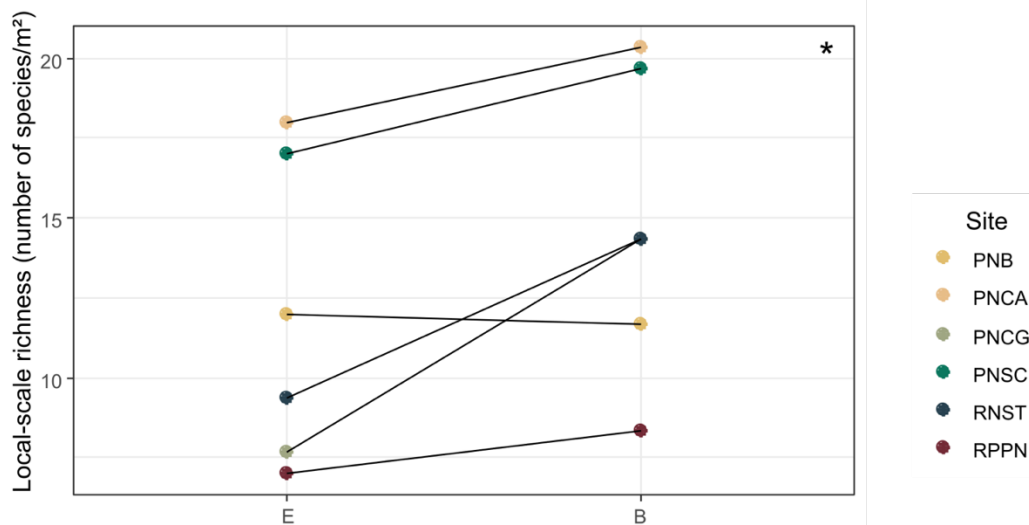


Figure 4. Species richness in fire-excluded (E) and in frequently and recently burned (B) tropical open ecosystems in Brazil: Parque Nacional de Brasília (PNB), Parque Nacional

dos Campos Amazônicos (PNCA), Parque Nacional da Chapada dos Guimarães (PNCG), Parque Nacional Serra do Cipó (PNSC), Reserva Natural Serra do Tombador (RNST) and Reserva Particular do Patrimônio Natural – Catedral do Jalapão (RPPN). (*) Indicates *P* value <0.05 for GLMM models: species richness ~ treatment (fire-excluded and burned) with site as random factor.

Within-transect beta diversity (i.e., dissimilarity among ten 1-m² plots per transect) was statistically greater in frequently burned compared to fire excluded sites (Fig.5, $p < 0.01$, $z = 2.059$, $R = 0.408$), showing to be 25% greater in frequently burned sites. Partitioning of beta diversity in both frequently burned and fire-excluded sites was similar; species replacement (turnover) accounted for 70% and 72%, respectively, and compositional difference (nestedness) accounted for 30% and 28%, respectively. Fire had significant effect in the amount of dissimilarity derived from the nestedness component (Fig.5C, $p = 0.02$), indicating that the plant community of fire-excluded savannas represents a nested subset of burned savannas in which fire promote the arrival of new species, and no loss of species.

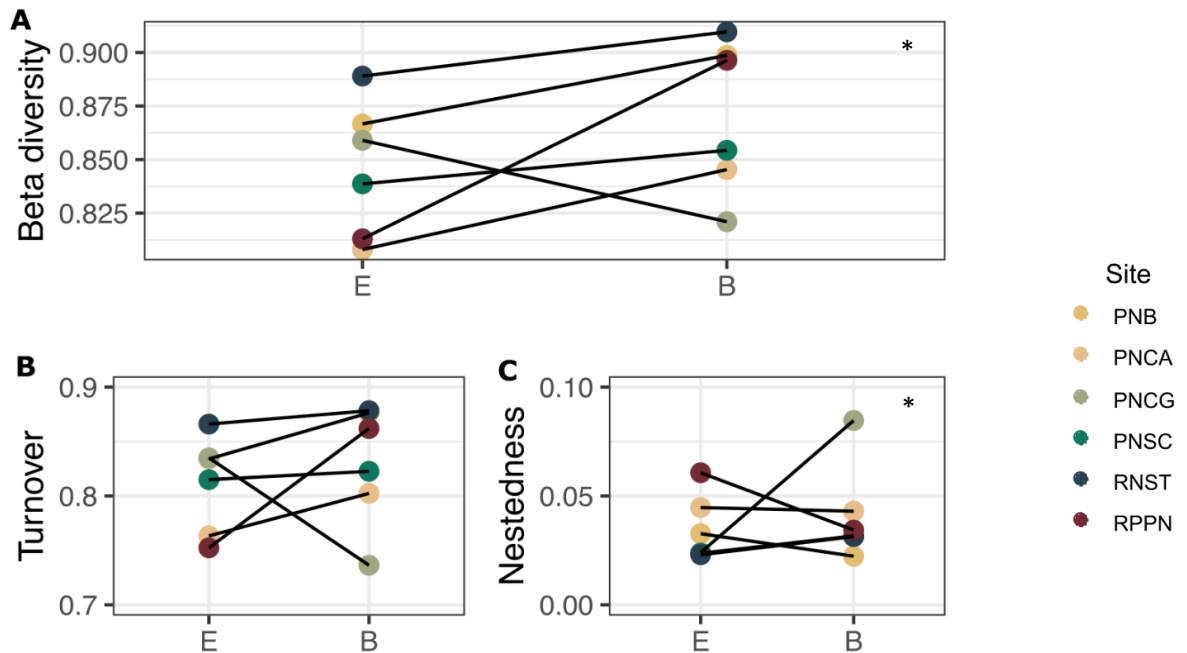


Figure 5. Beta diversity (A) and partitioning beta diversity into the components of turnover (B) and nestedness (C) in fire-excluded (E) and in frequently and recently burned (B) tropical open ecosystems in Brazil: Parque Nacional de Brasília (PNB), Parque Nacional dos Campos Amazônicos (PNCA), Parque Nacional da Chapada dos Guimarães (PNCG), Parque Nacional Serra do Cipó (PNSC), Reserva Natural Serra do Tombador (RNST) and Reserva Particular do Patrimônio Natural – Catedral do Jalapão (RPPN). (*) Indicates P value < 0.05 for GLMM models: beta diversity $\sim$ treatment (fire-excluded and burned) with site as random factor.

Analyses of aboveground biomass showed that fire-excluded sites had twice the accumulation of total aboveground biomass compared to frequently burned sites (Fig.6A, $p < 0.001$, $z = 4.430$, $R = 0.450$; see Table S3 – Supplementary information). Overall, total aboveground biomass ranged from 239 to 597 g.m^{-2} for frequently burned sites compared to 313 to 1360 g.m^{-2} for fire-excluded sites (see Table S3). Live biomass was no different between treatments (Fig.6B, Table S3, $p \geq 0.05$), while dead biomass (Fig.6C, Table S3, $p < 0.001$, $z = 4.950$, $R = 0.463$) was far higher in fire-excluded sites.

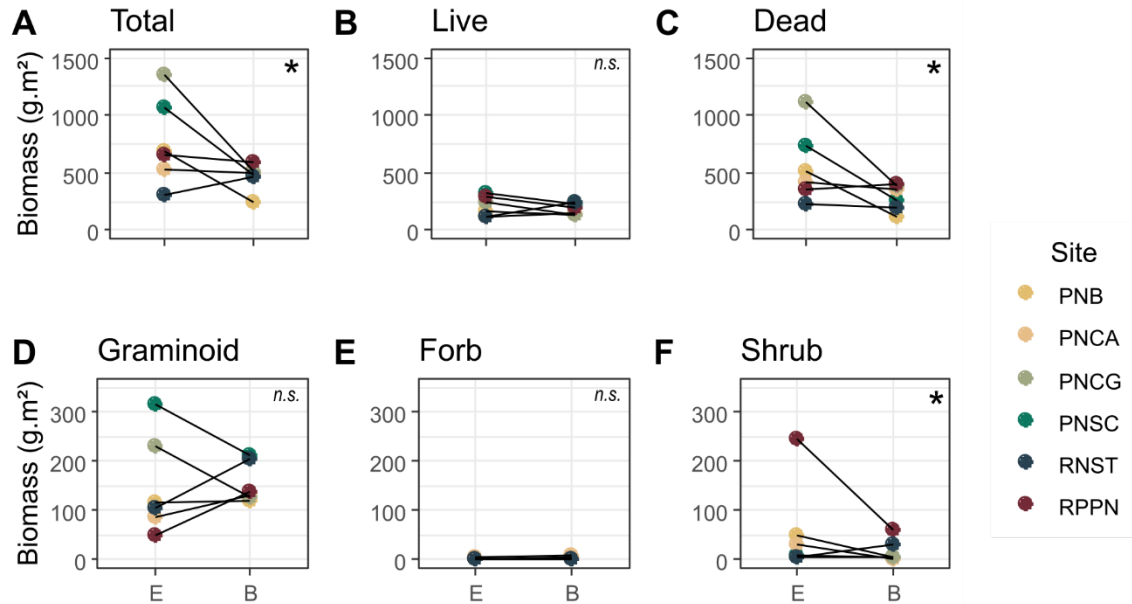


Figure 6. Total (A), live (B), dead aboveground biomass (C, g.m⁻²), and the growth forms components of live biomass: graminoids (D), forbs (E) and shrubs (F, g.m⁻²), sampled in fire-excluded (E) and in frequently and recently burned (B) tropical open ecosystems in Brazil: Parque Nacional de Brasília (PNB), Parque Nacional dos Campos Amazônicos (PNCA), Parque Nacional da Chapada dos Guimarães (PNCG), Parque Nacional Serra do Cipó (PNSC), Reserva Natural Serra do Tombador (RNST) and Reserva Particular do Patrimônio Natural – Catedral do Jalapão (RPPN). (*) Indicates *P* value < 0.05 for GLMM models: biomass ~ treatment (fire-excluded and burned) with site as random factor.

Aboveground live biomass was mostly composed of graminoids (about 90%) in both burned and fire-excluded savannas (Fig.6D, Table S4 – Supplementary information). We found no effect of fire exclusion on forb aboveground biomass, which reached a maximum of 9.6 g.m⁻² in burned savannas and represented, on average, 1,5% of aboveground live biomass (Fig.6E, Table S4).

Shrub biomass was 15% higher in fire exclude sites (Fig.6F, *p* < 0.001, *z* = 2.175, *R* = 0.391). Shrub biomass ranged from 3 to 62 g.m⁻² for frequently burned sites compared to 6 to 245 g.m⁻² for fire-excluded sites (Table S4).

Discussion

Despite the existence of several environmental filters (edaphic conditions, precipitation, climate, altitude) and vegetation types, our results indicate that fire is a driver of plant community structure and composition in tropical savannas. We found that fire increases local species richness and beta-diversity whereas it decreases biomass accumulation in savannas that are not yet experiencing woody encroachment.

Empirical and theoretical research have consistently supported the idea that fire shapes and maintains the herbaceous layer of tropical savannas (Abreu et al., 2017; Bond & Keeley, 2005; Fidelis et al., 2018; Durigan & Ratter, 2016). Our study adds empirical evidence to this increasing literature that savannas that do not burn are not constrained only due to forest expansion but also by the absence of fire. In all study locations, fire is an ecological filter that clearly distinguish frequently and recently burned from fire-excluded savannas according to the plant community composition leading a loss of species richness in fire-excluded savannas. We found change in species composition in the recently and frequently burned communities, showed to have higher species richness and beta diversity. Graminoids species showed to be higher in most of burned metacommunities with more new species able to establish after fire.

One of the main regeneration strategies that many species rely on to persist and resprout after fire is throughout the belowground bud bank (Bombo et al., 2022; Pilon, Cava, et al., 2021; Zupo et al., 2021). Once fire exclusion leads to a loss in the belowground bud bank density and diversity (Bombo et al., 2022), we would expect differences in the recruitment of forb species which rely mainly on the belowground bud bank to recover after fire (Bombo et al. 2022). In our study, two years after fire passage could be time enough for forb species to resprout, colonize the aboveground, and may no longer be found in the aboveground plant community by several ecological process, such as dry season (no water

available) or herbivory. In this scenario, forbs species may stay dormant through the belowground bud bank just waiting to be stimulated to resprout and colonize the aboveground plant community after a new fire passage. We suggest that this process explains the lack of difference in forb richness between burned and fire-excluded communities.

In fire-excluded savannas, we found more shrub biomass and total aboveground biomass, which is consistent with several studies (Durigan & Ratter, 2016; Rosan et al., 2019). Fire that reduces tree cover is essential for the herbaceous plant communities to keep their composition in savannas (Zimmermann et al., 2010) because it keeps canopies open and maintains graminoids and forbs species. Although our fire-excluded communities remain open areas with a continuous herbaceous layer and show no signs of encroachment process, we observed a higher accumulation of total aboveground biomass. This accumulation may lead to limitations in biotic recruitment, potentially producing negative effects on species-rich plant communities (Myers & Harms, 2009). In such environments, dominant species within large-stature guilds compete with rare species from small-stature guilds. This competition for resources and space, along with promoting diverse biotic interactions, can be crucial factors in determining species diversity within plant communities (Myers & Harms, 2009).

In conclusion, our results reinforce that although edaphic factors, climate conditions, and tree-grass interactions influence aspects of tropical savanna plant communities, fire is clearly a key ecological driver that directly maintains herbaceous layer diversity. Frequent fires enhance species richness and beta-diversity in tropical savannas. Conversely, suppressing fire leads to an accumulation of dead biomass which increases the risk of severe and intense wildfires, thereby threatening the resilience of tropical savannas. Therefore, frequent fires are essential for the conservation of these ecosystems and

underscores the importance of fire as a management tool in maintaining these open environments even it remains open independent of tree.

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Fire controls tropical savanna plant composition, diversity, and aboveground biomass independent of trees

Mariana Dairel, Tadeu Siqueira, Joseph Veldman, Alessandra Fidelis

Supplementary information

Table S1. General information about experimental design for sampling sites of open ecosystems including distance between treatments with different fire regimes (burned and fire-excluded) and distance between transects within each site. All distances were measure as a straight line. Abbreviation means: PNSC- Parque Nacional da Serra do Cipó; PNB- Parque Nacional de Brasília; PNCA- Parque Nacional dos Campos Amazônicos, PNCG- Parque Nacional da Chapada dos Guimarães; RNST- Reserva Natural Serra do Tombador and RPPN- Reserva Particular do Patrimônio Nacional Catedral do Jalapão.

Sites	Distance treatment (km)	Fire	Distance transects (T1-T2, m)	Distance transects (T2-T3, m)
PNSC	10	Burned	60	80
		Fire-excluded	30	30
PNB	5	Burned	15	15
		Fire-excluded	20	18
PNCA	15	Burned	80	76
		Fire-excluded	30	40
PNCG	10	Burned	52	54
		Fire-excluded	64	62
RNST	3	Burned	90	70
		Fire-excluded	50	52
RPPN	3	Burned	15	20
		Fire-excluded	30	35

Table S2. Mean (1m²) and total species richness (30m²) and species richness per growth form: G – graminoid, F – forb, and S- shrub sampled in both treatments, frequently burned (B) and fire-excluded (E), in different tropical open ecosystems in Brazil: Parque Nacional de Brasília (PNB), Parque Nacional dos Campos Amazônicos (PNCA), Parque Nacional da Chapada dos Guimarães (PNCG), Parque Nacional Serra do Cipó (PNSC), Reserva Natural Serra do Tombador (RNST) and Reserva Particular do Patrimônio Natural – Catedral do Jalapão (RPPN).

Site	Treatment	Mean richness (1m ²)				Total richness (30m ²)			
		Species richness	G	F	S	Species richness	G	F	S
PNB	B	11.6	6.5	4.2	0.9	50	17	25	8
	E	11.1	4.3	3.9	2.9	76	16	39	20
RPPN	B	8.3	4	2	2.3	25	8	10	7
	E	6.7	2.2	1.6	2.9	39	7	13	19
PNCA	B	20.3	7	11.7	1.6	57	21	32	4
	E	18.1	8.8	7.6	1.8	66	27	27	12
PNCG	B	16.5	9.8	5.9	0.8	63	21	27	12
	E	9.7	3.5	2.3	0.93	28	13	12	3
PNSC	B	19.7	12	6.8	0.9	87	39	41	7
	E	16.5	8.4	6.7	1.4	70	29	36	5
RNST	B	12.2	4.9	4.4	2.9	84	21	32	31
	E	9.3	4.7	2.5	2.1	68	18	25	25

Table S3. Total, live and dead aboveground biomass (g.m^{-2} , mean $\pm$ SE) sampled in both treatments, frequently burned (B) and fire-excluded (E), in different tropical open ecosystems in Brazil: Parque Nacional de Brasília (PNB), Parque Nacional dos Campos Amazônicos (PNCA), Parque Nacional da Chapada dos Guimarães (PNCG), Parque Nacional Serra do Cipó (PNSC), Reserva Natural Serra do Tombador (RNST) and Reserva Particular do Patrimônio Natural – Catedral do Jalapão (RPPN).

Site	Treatment	Total	Live	Dead
PNB	B	238.88 $\pm$ 31.91	129.82 $\pm$ 15.45	109.06 $\pm$ 22.23
	FE	688.46 $\pm$ 61.13	169.11 $\pm$ 28.46	519.35 $\pm$ 60.94
PNSC	B	486.01 $\pm$ 36.53	220.43 $\pm$ 25.19	265.58 $\pm$ 30.06
	E	1061.97 $\pm$ 134.04	328.50 $\pm$ 29.70	733.47 $\pm$ 112.63
PNCA	B	492.01 $\pm$ 46.15	142.92 $\pm$ 15.03	349.09 $\pm$ 34.82
	E	536.22 $\pm$ 54.91	121.66 $\pm$ 14.81	414.56 $\pm$ 44.76
PNCG	B	519.38 $\pm$ 72.29	132.24 $\pm$ 20.96	387.13 $\pm$ 55.29
	E	1357.97 $\pm$ 118.28	237.72 $\pm$ 23.73	1120.25 $\pm$ 101.91
RPPN	B	597 $\pm$ 105.44	199.06 $\pm$ 28.03	397.84 $\pm$ 81.11
	E	654.76 $\pm$ 177.11	295.32 $\pm$ 84.14	359.44 $\pm$ 105.30
RNST	B	467.78 $\pm$ 56.62	237.35 $\pm$ 35.80	200.47 $\pm$ 25.41
	E	313.22 $\pm$ 30.19	122.75 $\pm$ 12.98	230.43 $\pm$ 33.11

Table S4. Growth forms componentes of live biomas as graminoids, forbs and shurbs aboveground biomass (g.m^{-2} , mean $\pm$ SE) sampled in both treatments, frequently burned (B) and fire-excluded (E), in diferente tropical open ecosystems in Brazil: Parque Nacional de Brasília (PNB), Parque Nacional dos Campos Amazônicos (PNCA), Parque Nacional da Chapada dos Guimarães (PNCG), Parque Nacional Serra do Cipó (PNSC), Reserva Natural Serra do Tombador (RNST) and Reserva Particular do Patrimônio Natural – Catedral do Jalapão (RPPN).

Site	Fire regime	Graminoids	Forbs	Shrubs
PNB	B	119.72 $\pm$ 13.05	3.53 $\pm$ 1.36	6.58 $\pm$ 4.09
	E	116.84 $\pm$ 19.46	2.47 $\pm$ 0.86	49.80 $\pm$ 30.61
PNSC	B	212.98 $\pm$ 26.46	1.20 $\pm$ 0.42	6.25 $\pm$ 5.06
	E	315.41 $\pm$ 32.20	2.70 $\pm$ 0.39	10.39 $\pm$ 4.73
PNCA	B	130.42 $\pm$ 13.38	9.71 $\pm$ 1.10	2.78 $\pm$ 1.54
	E	85.40 $\pm$ 11.01	5.89 $\pm$ 1.56	30.37 $\pm$ 10.97
PNCG	B	126.19 $\pm$ 18.87	0.00 $\pm$ 0.00	6.06 $\pm$ 5.58
	E	228.72 $\pm$ 25.06	3.09 $\pm$ 3.09	5.91 $\pm$ 5.91
RPPN	B	137.03 $\pm$ 27.72	0.02 $\pm$ 0.02	62 $\pm$ 19.31
	E	50.01 $\pm$ 18.35	0.21 $\pm$ 0.21	245.10 $\pm$ 77.73
RNST	B	204.34 $\pm$ 32.02	2.52 $\pm$ 0.94	30.49 $\pm$ 10.51
	E	105.32 $\pm$ 10.99	1.60 $\pm$ 0.75	5.84 $\pm$ 2.34

Are tropical savannas resilient to fire? The example of the Cerrado

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Abstract

Fire is an important ecological factor influencing structure and diversity of tropical savannas. Therefore, fire exclusion can change plant community structure leading to losses in biodiversity and ecosystem services, being one of the major threats for the resilience of tropical savannas. In this study we aimed to analyze the plant community regeneration in tropical savannas with different fire frequencies (high x low), that were burned, in order to evaluate their resilience. We hypothesized that the lower the fire frequency is, the slower the vegetation regeneration will be. We established 30 plots in each site with distinct fire frequencies: I)- high-frequency fire (HF): 15 fires, excluded from fire for 7 years; II)- low-frequency fire (LF): 7 fires, excluded from fire for 15 years. Both sites have burned and unburned treatments. In burned treatments, we found that total biomass plots increased over time ($p < 0.001$) for both sites, while no differences were found in unburned ones. Live biomass in HF burned was about three times higher than LF burned, showing the same values as HF unburned one year after fire. The live biomass at LF burned increased over time ($p < 0.01$). The biomass composition was similar between treatments in both areas. The biomass was mostly composed of graminoids which represented 85% of live biomass in HF and LF for burned and unburned treatments. Fire increased graminoids at LF burned ($p < 0.01$). Further, HF showed higher species diversity ($H'_{\text{burned}}=2.44$ and $H'_{\text{unburned}}=2.40$) when compared with LF ($H'_{\text{burned}}=2.12$ and $H'_{\text{unburned}}=1.80$). Therefore, high fire frequency in tropical savannas is important to maintaining structure, composition, and diversity in plant communities and, moreover, guarantees their resilience.

1 Introduction

Disturbance-driven ecosystems, such as tropical savannas, are characterized by their dependence on recurring endogenous disturbances, like fire and herbivory, which play a crucial role in shaping their structure and dynamics (Bond & Keeley, 2005; Buisson et al., 2019). Plants from tropical savannas present a set of fire-adapted traits, ensuring their post-fire persistence and regeneration, thus maintaining biodiversity under suitable fire regimes (Keeley et al., 2011; Pausas & Keeley, 2014). However, these ecosystems are being rapidly lost not only due to agricultural expansion, and tree plantations, but also because of human-induced alterations in fire regimes, such as fire suppression (Buisson et al., 2019; Veldman et al., 2015).

Historically, tropical savannas have evolved under frequent natural fires (Simon et al., 2009), highlighting the role of fire in maintaining biodiversity, supporting unique species assemblages (Abreu et al., 2017), and shaping landscape-level patterns of vegetation distribution (Staver et al. 2011). The Cerrado is a highly biodiverse ecosystem encompassing several vegetation types, ranging from open to woody ecosystems, shaped by different fire regimes among other factors (Coutinho, 1982; Pivello, 2011). Changes in the fire regime, particularly due to fire suppression, leads to alterations in plant community structure, diminishing diversity and, potentially compromising the ecosystem resilience (Abreu et al., 2017; Buisson et al., 2019; Veldman et al., 2015).

Tropical savannas are resilient to fire (Bond 2004; Bond 2016; Buisson et al. 2019). However, variations on fire frequency in tropical savannas can lead plant communities with different composition, due to changes in species abundance and the substitution of species (Rodrigues & Fidelis 2022). The resilience of fire-driven systems under recurrent fires is dependent on site conditions and ecosystems type (Keeley & Zedler 1978; Bond & Keeley 2005; Buisson et al. 2019). In tropical savannas, resprouting from the belowground bud

bank is the most important mechanism for plants to recover after fire, conferring them high resilience to recurrent fire events (Pilon et al., 2021; Zupo et al., 2021). Fire exclusion negatively influences the bud-bearing belowground organs, diminishing the bud bank density, which can directly affect the ability of the systems to recover after fire (Bombo et al., 2022; Fidelis et al., 2014). Therefore, understanding how fire frequency and time since last fire affect the propagules source, in this case, the bud bank is crucial to realize how changes in fire regime will affect the resilience of tropical savannas.

This study evaluated how plant communities under different fire frequencies and time since last fire would respond to a fire event. We aimed to address the following questions: I) Do tropical savannas with low and high fire frequencies differ in species compositions? II) Is plant regeneration similar between tropical savannas with different fire frequencies? We hypothesize that plant community composition, structure, and diversity will differ between areas with different fire frequencies: areas under lower fire frequency will show lower diversity than areas under high fire frequency, with lower aboveground biomass. Since areas under lower fire frequency present a decline in bud bank density (Bombo et al. 2022), regeneration will be slower, with an increase in aboveground biomass, and no increases in diversity in comparison to areas where the bud bank is still viable. These areas might have plant communities with a higher proportion of fire-sensitive species and thus, fire would have a negative effect on them. As a consequence, tropical savannas with frequent fires rapidly recover their aboveground plant community following fire events. Also, these areas will show no changes in species richness after fire, but we will probably see short-term changes in the species composition, due to the emergence of dormant species from the bud bank. High frequent fire sites would also have a higher post-fire accumulation of aboveground biomass, mainly grasses and forbs, since fire might stimulate buds to produce aboveground shoots. Therefore, areas under high frequent fires would be

more resilient to fire, even after 10 years of fire exclusion, than areas under lower fire frequency. Understanding the resilience of tropical savannas due to environmental changes and managements interventions, including the effects of past burning legacies, is essential for guiding effective restoration efforts (Buisson et al., 2019, 2020; Lyons et al., 2023; Silveira et al., 2020), prioritizing biodiversity conservation and ecosystem services in tropical savannas.

2 Materials and Methods

2.1. Study area

The study was carried out at the Reserva Natural Serra do Tombador (RNST, 47°45'–51' W and 13°35'–38' S, 8900 ha, 560–1118 m) located in Central Brazil. The climate is tropical, highly seasonal with a well-marked dry season (May to September), when the accumulated precipitation is lower than 60 mm, and the rainy season (October to April) when most of the annual precipitation occurs ranging from 1300-1500 mm/year. The mean annual temperature ranges from 20 to 25°C, recording minimum temperatures of 15°C and higher temperatures up to 36°C (Fundação Grupo Boticário, 2011).

The region of RNST has a history of anthropogenic and natural fires (lightning) with a mean fire return of three years (Daldegan et al., 2014) until 2006 when the preserve was created. Since 2006, most of the areas were excluded from fire, although wildfires would eventually occur. In 2017, the dry season extended until mid-end of October. The long dry season together with the high accumulation of fuel load, wildfires occurred at the RNST, burning about 90% of the entire protected area (Fidelis et al., 2018).

After the wildfires, sites that had previously different fire frequencies (Alves et al. in preparation) were selected to establish this study. We chose two sites that were excluded from fire for at least seven years and burned during the wildfires in 2017: I)- High-frequency fire sites

(HF): 15 fire events in the last 30 years; II) Low-frequency fire sites (LF): seven fire events in the last 30 years. For both sites, HF and LF, we have burned x unburned treatments, where we could establish our sampling plots. The unburned areas at HF burned the last time in 2011 (seven years of exclusion), while at LF the last fire occurred in 2002.

At the RNST, the vegetation is comprised of a mosaic of vegetation types, ranging from grassy to woody ecosystems (Fundação Grupo Boticário, 2011), which is shaped among other factors by distinct fire regime (Daldegan et al., 2014). Both of our study sites (HF and LF) are open savannas characterized by a continuous species-rich herbaceous layer, mainly composed by C4 grasses and forbs, with scattered shrubs and dwarf trees (Coutinho, 1978). At HF, the herbaceous layer is most dominated by perennial C4 grasses, which are the main component forming the continuous fuel load bed (Rodrigues et al., 2021). In LF, despite the dominance of the herbaceous layer, shrub and tree cover are notable higher compared to the HF site (personal observation).

2.2. Experimental design and data sampling

Aboveground biomass assessment

In each site (HF and LF) and treatment (burned and unburned) we randomly sampled the aboveground biomass in 10 plots 0.25m^2 (2 sites x 2 treatments x 10 plots/treatment, total of 40 plots). The total aboveground biomass (live + dead biomass) was clipped at the ground level in each 0.25m^2 plot and taken to the lab to be sorted according to the following components: dead biomass, graminoids, forbs and shrubs (<1.5 m). Aboveground biomass was then oven-dried at 80°C for 48h and weighed. Litter collected on the soil surface was also oven-dried in the same conditions and then weighed. Hereafter, the component dead biomass is the sum of dead standing biomass and litter. We sampled aboveground biomass in the rainy season for two years after fire (2018 and 2019).

System productivity for burned treatments was assessed throughout the aboveground biomass data collected in the rainy season. The productivity of the first year after fire (2018) was considered the total amount of biomass sampled in burned plots. Productivity for 2019 was the difference in total aboveground biomass accumulated between 2019 and 2018. Productivity was calculated for total aboveground biomass and the live biomass according to each functional group: graminoids, forbs and shrubs.

Plant Community survey

To assess plant community composition and structure, we randomly established 30 1m² permanent plots, within a minimum distance of 5m among plots, in each site (HF and LF) and treatment (burned and unburned, 2 sites x 2 treatments x 30 plots/treatment, total of 120 plots). In each 1m² plot, we visually estimated the percentage cover of bare soil, dead biomass (litter + standing dead biomass) and plant species. The visual estimation was conducted using categories of 10%, except for the categories 1% and 5%. All species were identified at species level when it was possible. Plant species were classified into three growth forms: graminoids (grasses and sedges), forbs and shrubs (<1.5m height). The plant community survey was carried out four times in the period of two years after fire: rainy season (January 2018 and 2019) and dry season (August 2018 and October 2019).

2.3. Data analyses

We used general linear models (GLM, fixed factor: site, random effect: plot, family: Gaussian) to evaluate whether different fire frequency influenced the aboveground biomass components and biomass productivity after fire in both LF and HF. We assessed

statistical differences of aboveground biomass components between treatments and years within sites by performing Tukey test. To evaluate the pairwise aboveground biomass components in each site (HF and LF) with different treatments (burned and unburned) and years we used pairwise post-hoc tests of HSD Tukey. Moreover, we performed a Tukey test to obtain differences in productivity within burned treatment across two consecutive years after fire (2018 and 2019). Analyses were carried out separately for each site (HF and LF).

To investigate species composition between treatments (burned and unburned) within sites (HF and LF) we performed Principal Coordinate Analysis (PCoA) to conduct trajectory analysis based on two data matrices: I)- described as species cover in each observation, using Bray-Curtis distance as a resemblance measure; II)- described as species presence-absence in each survey time, using Jaccard distance. To compare plant community composition between treatments (burned and unburned) for each site (HF and LF), we performed permutational multivariate variance analysis (PERMANOVA), using *'strata'* as a function argument to constrain permutations within sites. Further, we compared two diversity metrics, species richness and Shannon diversity, between treatments within sites using *iNEXT* function. Combined with diversity metrics, we calculated partitioning beta diversity into turnover and nestedness components.

Analyses were performed using the R software version 4.2.2 (R Development Core Team, 2022) using the packages: *emmeans* (Lenth, 2021), *Ecotraj* (De Caceres et al, 2019), *dplyr* (Wickham et al, 2023), *vegan* (Oksanen et al., 2020), *pairwiseAdonis* (Martinez, 2019), *iNEXT* (Hsieh et al., 2020), *betapart* (Baselga et al. 2020) and *ggplot2* (Wickham, 2016).

3 Results

Aboveground biomass and productivity

Aboveground biomass was able to rapidly regenerate independently of fire frequency, reaching about 85% and 92% recovery two years post-fire in high-frequency (HF) and low-frequency (LF) fire sites, respectively (Fig. 1A, Supplementary material – Table S1). Overall, total biomass in unburned treatment was significantly higher than burned treatments within year in HF and LF sites ($p < 0.05$, Fig. 1A, Supplementary material – Table S1, S2), mainly due to the high accumulation of dead biomass ($p < 0.001$, Fig. 1B). LF sites in the second year post-fire, however, showed a higher accumulation of total biomass in burned plots in comparison to unburned ones, mostly due to the higher accumulation of live biomass (Supplementary material – Table S1, S2). However, unburned treatment showed no significant difference in total biomass accumulation over years, in both HF and LF sites (Fig. 1A, Supplementary material – Table S2).

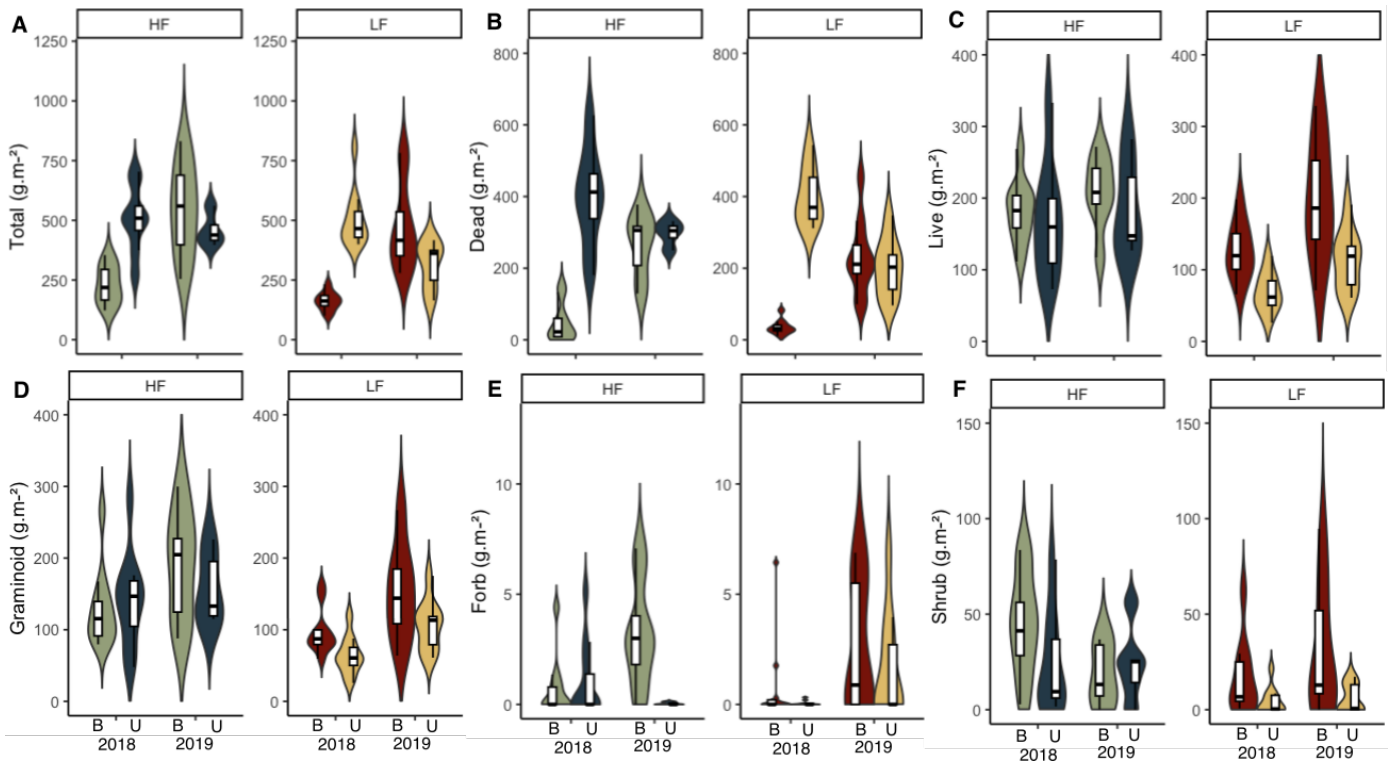


Figure 1. Total (A), dead (B), live (C) and growth forms aboveground biomass (g.m^{-2} , mean $\pm$ SE): graminoid (D), forb (E), and shrub (F) sampled in two sites under different fire frequencies that have been long-term fire excluded: high-

201 frequency fire (HF) and low-frequency fire (LF) for both burned and unburned treatments in one (2018) and two (2019)
202 years after fire in tropical savannas at Reserva Natural Serra do Tombador (RNST).

203

204 Live biomass accounted for 30% to 25% of the total biomass in HF and LF sites,
205 respectively (see Supplementary material – Fig. S1). HF unburned showed two- to three
206 -fold more live biomass compared to LF unburned. After fire, live biomass in HF and LF
207 fully recovered one year after fire ($p=0.4$; Fig. 1C, Supplementary material – Table S1,
208 S2). LF burned showed a significant increase in live biomass over time, being two times
209 greater in the second-year post-fire compared to the first one ($p<0.05$, Fig. 1C,
210 Supplementary material – Table S1, S2). Moreover, live biomass at LF unburned were
211 significantly higher between 2018 and 2019 (Fig. 1C, Supplementary material – Table
212 S1, S2).

213 The percentage of different growth forms (graminoid, forb and shrub) to the live
214 biomass was similar between burned and unburned treatments for both HF and LF sites
215 (Fig. 1D-F, Supplementary material – Table S3). Graminoid accounted for 78% and 86%
216 of live biomass (Supplementary material – Fig. S1) for both sites HF and LF,
217 respectively and did not differ between burned and unburned treatments within each site
218 (Fig. 1D, Supplementary material – Table S2). However, in LF sites that were burned,
219 graminoid biomass increased significantly over time ($p<0.05$, Fig. 1D, Supplementary
220 material – Table S2). Forb represented 2% of the live biomass (see Supplementary
221 material – Fig. S1) and did not differ between burned and unburned treatments for both
222 HF and LF sites (Fig. 1E, Supplementary material – Table S2). However, when compared
223 for each treatment over year (burned 2018-2019 and unburned 2018-2019), forb
224 biomass showed a significant increase in HF ($p<0.05$, Fig. 1E, Supplementary material –
225 Table S2). Shrubs accounted for 20% of the live biomass in HF and showed a higher
226 amount of biomass in burned treatment in the second-year post-fire compared to the

unburned treatment ($p=0.01$, Fig. 1E). On the other hand, in LF, shrub biomass represented 12% of the live biomass and did not differ between burned and unburned treatments ($p>0.05$, Fig. 1E, Supplementary material – Table S2). Shrub biomass presented a 3% increase in burned treatment between the first and second-year post-fire (Fig. 1E).

The productivity in burned treatments did not differ between years at sites with high fire frequency (HF, Fig.2A, Supplementary material – Table S4), being 30% higher than in LF burned. At HF sites, live biomass productivity was significantly higher in the first rainy season after fire ($p=0.005$, Fig. 2B), which was driven mainly by the high productivity of graminoids ($p=0.002$, Fig.2C) that represented 70% of the live biomass productivity. Forb productivity was higher in the second-year post-fire ($p=0.01$, Fig.2D) accounting for <1% of the live biomass productivity at HF sites. In LF sites, total productivity differed between years ($p=0.007$, Fig. 2A), drive mainly for the decrease of shrub productivity two years after fire ($p=0.02$, Fig.2E).

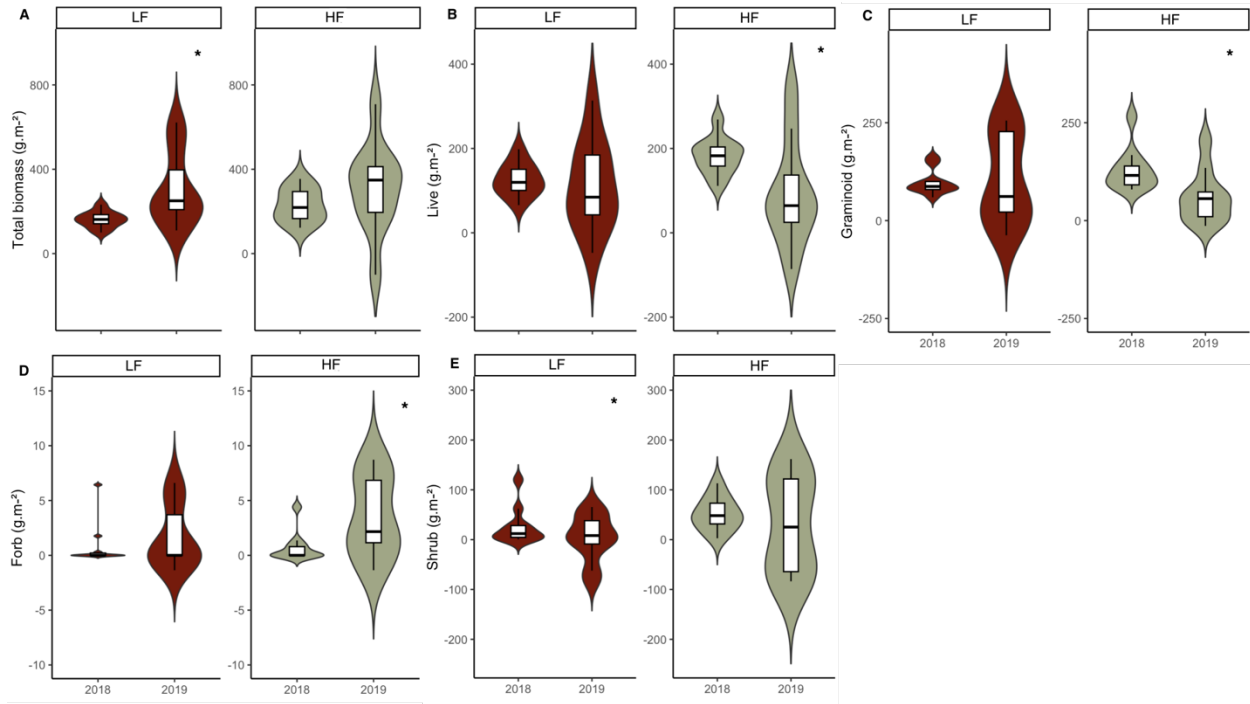


Figure 2. Productivity of total biomass (A), live biomass (B) and for the growth forms: graminoid (C), forb (D) and shrub (E) sampled in two sites under different fire frequencies that have been long-term fire excluded: high-frequency fire (HF) and low-frequency fire (LF) for burned treatment in one (2018) and two (2019) years after fire in tropical savannas at Reserva Natural Serra do Tombador (RNST).

Plant community composition and richness

Plant community cover in HF and LF sites was significantly affected by fire. Dead biomass cover increased over time in both sites ($p < 0.001$, Fig. 3A, Supplementary material – Table S5), with 45% higher cover of dead biomass in unburned treatments in both sites (HF and LF). Conversely, burned treatments presented ca. 30 to 40% higher bare soil cover in HF and LF sites, respectively (Fig. 3B). Bare soil cover showed a decrease over time ($p < 0.05$, Supplementary material – Table S5), except for LF burned which maintained a higher bare soil cover (Fig. 3B). At both HF and LF sites, growth forms cover (graminoid, forb and shrub) did not significantly differ between burned and unburned treatments (Fig. 3C-E, Supplementary material – Table S5). However, shrub percentage cover was significantly higher after fire in LF burned (Fig. 3E, Supplementary

material – Table S5). Nevertheless, HF burned and LF burned presented the same percentage cover of graminoids after fire (Fig.3C).

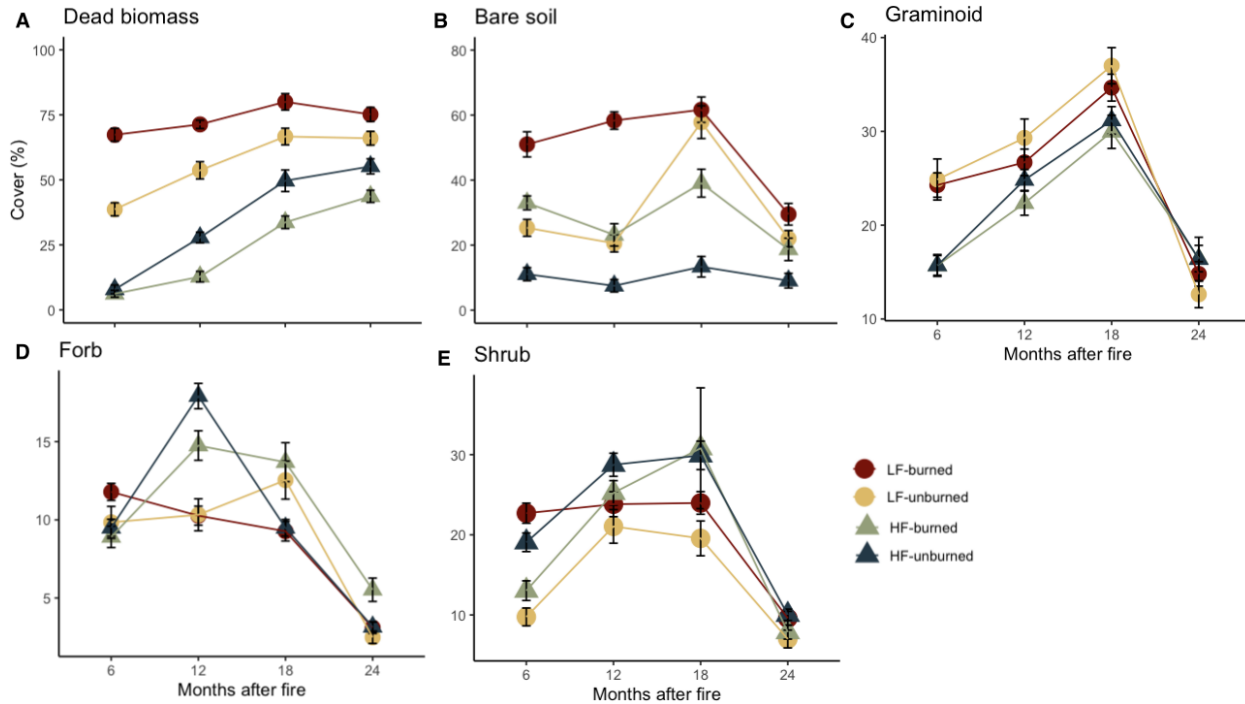


Figure 3. Trajectories of plant communities components: dead biomass (A), bare soil (B) and growth forms: graminoid (C), forb (D), and shrub (E) by their ground cover (%) for 6, 12, 18 and 24 months after fire sampled in two sites under different fire frequencies that have been long-term fire excluded: high-frequency fire (HF) and low-frequency fire (LF) for both burned and unburned treatment in tropical savannas at Reserva Natural Serra do Tombador (RNST).

Plant communities in high-frequency (HF) and low-frequency (LF) fire sites differed according to species composition for both species abundance ($F=5.53$, $p<0.0001$, Table 1) and presence-absence ($F=4.93$, $p<0.0001$, Table 1, Figure. 4). Regardless of the treatment, HF showed higher species richness and Shannon diversity compared to LF (Table 2). Additionally, burned treatments had greater species richness and Shannon diversity compared to unburned treatments for both sites, HF and LF (Table 2). *Betapart* analysis according to presence-absence of species revealed higher dissimilarities in species composition between LF burned and unburned compared to HF burned and unburned (Table 3). Partitioning into turnover and nestedness components, beta-diversity was better explained by turnover in both sites, with higher turnover observed at LF (Table 3).

Table 1. PERMANOVA analysis of plant communities sampled in two sites under different fire frequencies that have been long-term fire excluded: high-frequency fire (HF) and low-frequency fire (LF) by their cover (Bray-Curtis) and species composition (Jaccard) between burned and unburned treatments in tropical savannas at the Reserva Natural Serra do Tombador (RNST).

Pairs	Site	Dissimilarity measure	Dissimilarity	P value
Burned-Unburned	HF	Bray-Curtis	0.355	0.001
Burned-Unburned	LF	Bray-Curtis	0.577	0.001
Burned-Unburned	HF	Jaccard	0.354	0.001
Burned-Unburned	LF	Jaccard	0.589	0.001

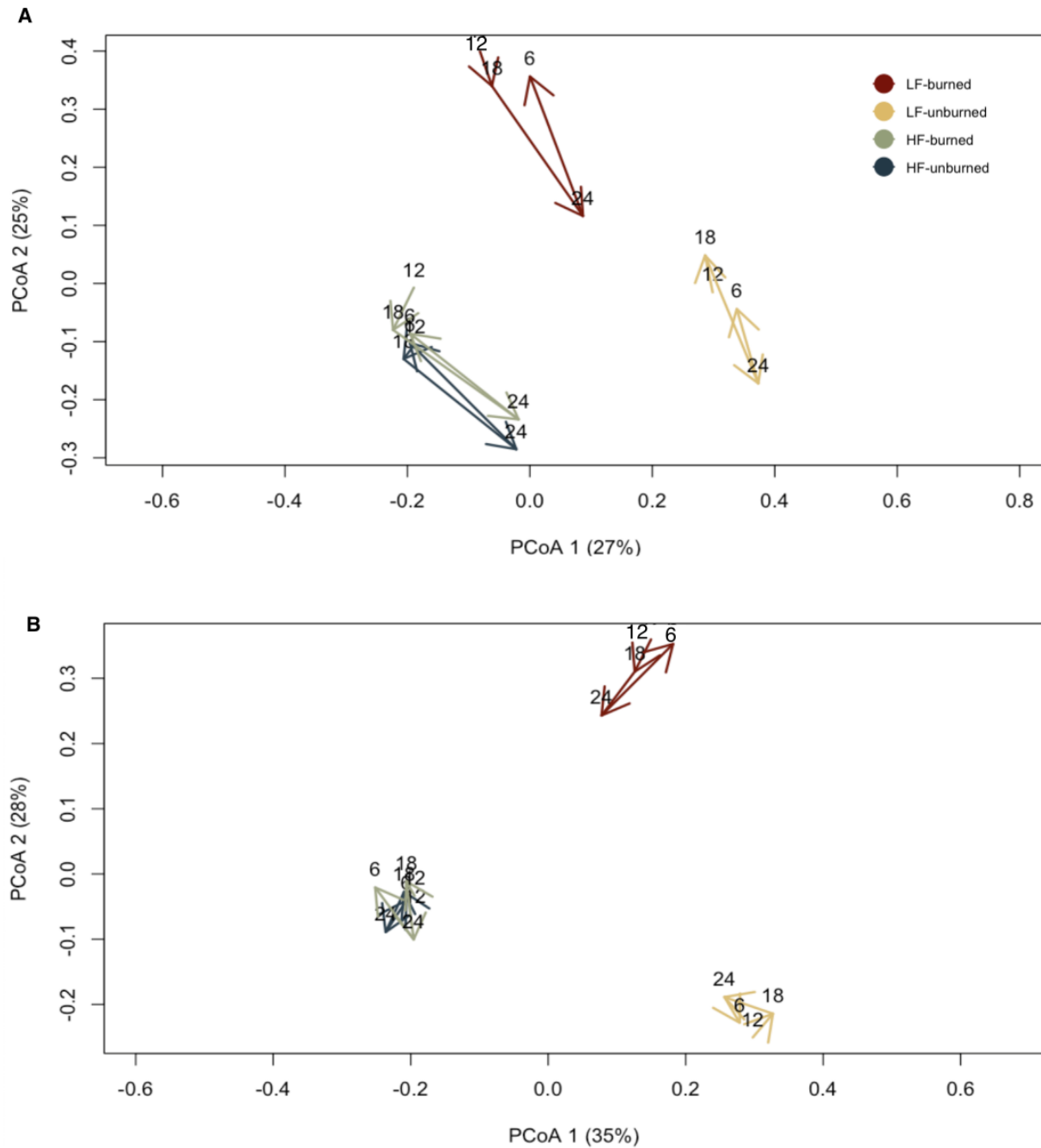


Figure 4. Trajectory principal coordinates analysis (PCoA) according to plant community composition described by species abundance (A) and species composition by presence/absence (B) for 6, 12, 18 and 24 months after fire from sites under different fire frequencies that have been long-term fire excluded: high-frequency fire (HF) and low-frequency fire (LF) for both burned and unburned treatments in tropical savannas at Reserva Natural Serra do Tombador (RNST).

Table 2. Richness (total number of species and number of species per m²) and Shannon diversity of plant communities sampled in two sites under different fire frequencies: high-frequency fire (HF) and low-frequency fire (LF) for both burned and unburned treatments in tropical savannas at Reserva Natural Serra do Tombador (RNST).

Site	Treatment	Species richness (Total)	Species richness (m ²)	Shannon diversity
HF	Burned	91	18	2.44
	Unburned	87	16	2.40
LF	Burned	90	13	2.13
	Unburned	67	9	1.80

Table 3. Partitioning beta-diversity into turnover and nestedness components of plant communities sampled in two sites under different fire frequencies: high-frequency fire (HF) and low-frequency fire (LF) between burned and unburned treatments in tropical savannas at Reserva Natural Serra do Tombador (RNST).

Site	Pairs	Turnover	Nestedness	P value
HF	Burned-Unburned	0.305	0.049	0.001
LF	Burned-Unburned	0.465	0.123	0.001

4 Discussion

Fire exclusion for long periods together with low fire frequency leads to changes in plant communities' structure and composition, as we observed in our study: LF sites had plant communities with different composition than HF sites (both abundance and presence-absence of species). Since tropical savannas are adapted and resilient to fire, one could expect that if LF sites would burn, we would be able to get back to the state, where plant communities were more diverse such as the HF sites. However, that was

not the case: once fire occurred in LF sites, plant communities' trajectories were different from both HF sites (burned and unburned) and LF unburned sites. Burned LF sites had communities that were not similar to their unburned ones: where we had higher species richness, turnover and higher cover of graminoids in the aboveground plant community. However, they still differed from the HF sites. This shows us that a threshold has already been crossed, probably due to the loss of bud-bearing belowground organs of species that need frequent fires, such as the ones with xylopodia (Fidelis et al., 2014; Bombo et al., 2022). Thus, burning long-term fire excluded sites that had low fire frequency does not guarantee the return to the state similar to sites of high fire frequency and shorter fire exclusion.

However, biomass amounts were not affected in a short-term after fire, vegetation still can recover its total biomass, although some differences in how it recovers could be observed: higher percentage of shrub biomass in HF site and higher percentage of graminoids biomass in LF site. Burned HF sites had higher graminoid and forb productivity. Is well-known that grasses may have a good response to fire (Simpson et al., 2019) where high-frequency fire selects for more vigorous post-fire plant community recruitment, inducing greater resprouted biomass (Hadley & Kieckhefer, 1963; Moreira et al., 2012; Simpson et al., 2019). Moreover, high-frequent fires promote greater bud bank diversity and density (Bombo et al., 2022) from which mostly forb species are dependent to recover after fire. However, LF burned had a decrease in shrub productivity in the short-term after fire, probably due to the decrease of bud bank organs in these sites (Bombo et al., 2022) or to the shift of plant community composition from fire-resistant species to fire-sensitive ones.

Fire exclusion leads to high accumulation of aboveground biomass mostly driven by the high amount of dead biomass (Zupo et al., 2022), which is an important driver to

the grass-fire cycle (D'Antonio & Vitousek, 1992). The aboveground plant communities of both sites were dominated by graminoids in the herbaceous layer, comprising more than 70% of the plant community. Both high-frequency (HF) and low-frequency fire (LF) sites presented a high amount of dead biomass before fires, which may have resulted in fires of higher intensity and severity (Hiers et al., 2020). However, biomass recovery was at similar rates in HF and LF sites (85% and 92%, respectively), showing the great capacity of graminoids to regenerate after fire, because they still keep their bud banks for longer periods (Bombo et al. 2022). Moreover, we could observe that, even after fire, both areas begin to rapidly accumulate dead biomass again, which should be an important point to be addressed for the elaboration of fire management, since the high accumulation of dead biomass can lead to wildfires. Overall, it is important to call the attention of their implementation of fire management in the correct seasons and frequencies (de Souza et al., 2023; Schmidt et al., 2018b) controlling the accumulation of fuel load avoiding big wildfires (Fidelis et al., 2018) and promoting a mosaic of plant communities with different assemblages and structure.

Before savannas transitions towards a close state, graminoids increase in cover and biomass, and this is also reflected in the belowground components of savannas, since more rhizomes are found in these areas, guaranteeing their post-fire resprouting (Bombo et al., 2022). Although the recovery of forb and shrub species is slower in sites under low fire frequency because these species rely on the belowground bud bank and most of the bud-bearing structure as xylopodia disappear from the belowground community (Bombo et al., 2022), reflecting the slower recovery of these species in short-term after fire. Overall, our results showed that the first rainy season post-fire might be a key factor for plant community recovery over time in tropical savannas, not only for total biomass but also for different growth forms components from the live biomass.

The similarity in plant community composition between burned and unburned under high frequency fire showed that seven years of fire exclusion have not been enough to change the plant community structure and species composition. Thus, plant communities under HF are still resilient to fire. Both above and belowground community are still able to promote plant recovery after fire (Bombo et al., 2020). However, communities under lower fire frequency and higher fire-exclusion were not able to reach the same composition and richness as HF sites after fire, showing that these communities already lost typical species from frequent burned savannas. Apart from changes in the belowground community, the burned LF site might also undergo a transition of state from grassy to woody systems, and thus, changes from fire-resistant to fire-sensitive species may occur, leading to a loss of resilience to fire.

Our results bring important implications: fire management in long-term fire exclusion may not be efficient in recovering plant diversity and composition in tropical savannas, due to the loss of bud-bearing organs belowground and shifts from fire-resistant to fire-sensitive species. It may be that, keep burning these sites would promote higher plant diversity. However, an input from propagules from outside would be necessary, since we know that the belowground plant community suffered the loss of important bud-bearing organs. In addition, many Cerrado species produce a high number of empty propagules (Dairel & Fidelis, 2020) and tend to form a transient seed bank (Dairel & Fidelis, 2020b, Aires et al. 2014) which makes seedling recruitment harder to occur. Therefore, maintaining fire under a higher frequency in tropical savannas guarantees their resilience. There is no doubt about the positive effects of fire in maintaining structure, composition, and diversity in tropical savannas, therefore, we highlight the importance of using fire as a management tool and establishing appropriate

conservation policies once reverting the consequences of fire exclusion in tropical savannas is still a challenge.

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Are tropical savannas resiliente to fire? The example of the Cerrado

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Supplementary Information

Table S1. Total, live and dead aboveground biomass (g.m^{-2} , mean $\pm$ SE) sampled in two sites under different fire frequencies that have been long-term fire excluded: high-frequency fire (HF) and low-frequency fire (LF) for both burned and unburned treatments in one (2018) and two (2019) years after fire in tropical savannas at Reserva Natural Serra do Tombador (RNST).

Year	Site	Treatment	Total	Live	Dead
2018	HF	Unburned	597.3 $\pm$ 73.01	165.84 $\pm$ 24.45	431.46 $\pm$ 53.37
	HF	Burned	229.38 $\pm$ 25.06	182 $\pm$ 13.95	47.38 $\pm$ 17.01
	LF	Unburned	504.8 $\pm$ 38.59	68.45 $\pm$ 8.67	436.35 $\pm$ 42.90
	LF	Burned	162.2 $\pm$ 12.12	126.03 $\pm$ 12.36	35.17 $\pm$ 6.45
2019	HF	Unburned	568.75 $\pm$ 113.69	185.3 $\pm$ 30.10	383.46 $\pm$ 87.08
	HF	Burned	483.46 $\pm$ 59.62	186.72 $\pm$ 36.28	296.75 $\pm$ 27.03
	LF	Unburned	313.22 $\pm$ 30.19	112.75 $\pm$ 12.98	200.47 $\pm$ 25.41
	LF	Burned	467.78 $\pm$ 56.42	237.35 $\pm$ 35.80	230.43 $\pm$ 33.11

Table S2. Results of Tukey test and pairwise comparisons from general linear models analysis of aboveground biomass components (total biomass, dead biomass, live biomass and growth forms: graminoids, forb and shrub, g.m²) sampled in two sites under different fire frequencies that have been long-term fire excluded: high-frequency fire (HF) and low-frequency fire (LF) for both burned and unburned treatments in one (2018) and two (2019) years after fire in tropical savannas at Reserva Natural Serra do Tombador (RNST).

Component	Site	Pairwise	Pairwise	Pairwise	Pairwise
		Burned –	Burned –	Burned	Unburned
		Unburned	Unburned	2018 – 2019	2018 – 2019
		2018	2019		
Total biomass	HF	0.01	0.1	0.005	0.08
	LF	0.03	0.01	0.001	0.72
Dead biomass	HF	0.0001	0.06	0.0001	0.12
	LF	0.001	0.97	0.001	0.77
Live biomass	HF	0.39	0.39	0.13	0.18
	LF	0.0006	0.0006	0.003	0.0001
Graminoid	HF	0.99	0.69	0.23	0.34
	LF	0.005	0.001	0.001	0.001
Forb	HF	0.99	0.99	0.009	0.01
	LF	0.58	0.86	0.09	0.09
Shrub	HF	0.06	0.01	0.64	0.79
	LF	0.2	0.7	0.11	0.99

Table S3. Growth forms components of live biomass as graminoid, forb and shrub aboveground biomass (g.m^{-2} , mean $\pm$ SE) sampled in two sites under different fire frequencies that have been long-term fire excluded: high-frequency fire (HF) and low-frequency fire (LF) for both burned and unburned treatments in one (2018) and two (2019) years after fire in tropical savannas at Reserva Natural Serra do Tombador (RNST).

Year	Site	Treatment	Graminoid	Forb	Shrub
2018	HF	Unburned	141.35 $\pm$ 20.92	0.98 $\pm$ 0.56	23.51 $\pm$ 8.04
	HF	Burned	129.56 $\pm$ 17.45	0.73 $\pm$ 0.44	51.71 $\pm$ 10.5
	LF	Unburned	63.9 $\pm$ 8.45	0.7 $\pm$ 0.3	27.32 $\pm$ 11.9
	LF	Burned	129.56 $\pm$ 15.07	2.53 $\pm$ 1.09	23.16 $\pm$ 10.37
2019	HF	Unburned	157.68 $\pm$ 22.34	3.33 $\pm$ 2.02	24.29 $\pm$ 9.25
	HF	Burned	189.41 $\pm$ 23.5	4.22 $\pm$ 0.96	81.65 $\pm$ 22.29
	LF	Unburned	105.32 $\pm$ 10.99	1.6 $\pm$ 0.75	5.84 $\pm$ 2.34
	LF	Burned	204.34 $\pm$ 32.02	2.52 $\pm$ 0.94	30.49 $\pm$ 10.51

Table S4. Results of Tukey test and pairwise comparisons from general linear models' analysis of plant communities' components (total biomass, live biomass, graminoid, forb and shrub) by their productivity sampled in two sites under different fire frequencies that have been long-term fire excluded: high-frequency fire (HF) and low-frequency fire (LF) for burned treatment in one (2018) and two (2019) years after fire in tropical savannas at Reserva Natural Serra do Tombador (RNST).

Component	Site	Pairwise
		Burned 2018 - 2019
Total biomass	HF	0.1
	LF	0.007
Live	HF	0.005
	LF	0.67
Graminoid	HF	0.002
	LF	0.8
Forb	HF	0.01
	LF	0.32
Shrub	HF	0.5
	LF	0.02

Table S5. Results of Tukey test and pairwise comparisons from general linear models' analysis of plant communities components (dead biomass, bare soil, graminoid, forb and shrub) by their ground cover (%) sampled in two sites under different fire frequencies that have been long-term fire excluded: high-frequency fire (HF) and low-frequency fire (LF) for both burned and unburned treatments in one (2018) and two (2019) years after fire in tropical savannas at Reserva Natural Serra do Tombador (RNST).

Component	Site	Pairwise	Pairwise	Pairwise	Pairwise
		Burned –	Burned –	Burned	Unburned
		Unburned	Unburned	2018 – 2019	2018 – 2019
		2018	2019		
Dead biomass	HF	0.0001	0.0001	0.0001	0.0001
	LF	0.0001	0.0001	0.0001	0.0001
Bare soil	HF	0.0001	0.0001	0.0001	0.0001
	LF	0.0001	0.001	0.0001	0.0001
Graminoid	HF	0.94	0.34	0.18	0.18
	LF	0.82	0.82	0.56	0.15
Forb	HF	0.98	0.27	0.97	0.97
	LF	0.99	0.99	0.92	0.92
Shrub	HF	0.98	0.91	0.88	0.94
	LF	0.71	0.17	0.03	0.2

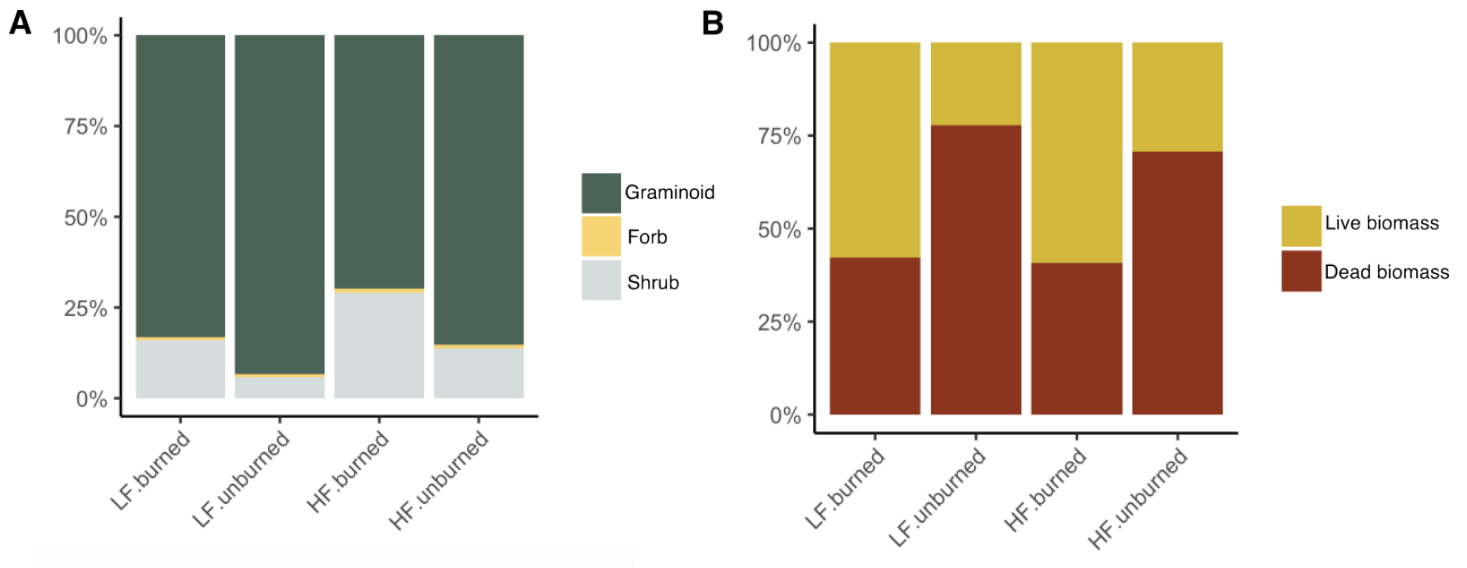


Figure S1. Percentage composition of aboveground biomass by growth forms (graminoid, forb and shrub; A), as well as live and dead biomass (B) sampled in tow sites under different fire frequencies that have been long-term fire excluded: high-frequency (HF) and low-frequency fire (LF) for both burned and unburned treatments in tropical savannas at Reserva Natural Serra do Tombador (RNST).

1 **How does fire regime shape regenerations strategies in tropical savannas?**

2 Running title: post-fire regeneration strategies in tropical savannas

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16

Abstract

1. In tropical savannas, fire is a common endogenous disturbance that maintains biodiversity under an appropriate fire regime. In these systems, plants have fire-adaptive traits that assure their survival and persistence after fires, guaranteeing their resilience. Aboveground plant communities regenerate fire through resprouting or seedling recruitment, and changes in fire regime can affect the way plants regenerate in the post-fire environment. The post-fire regeneration is therefore not only influenced by the fire regime, as well as by availability of the regeneration source (seed x bud bank).

2. We investigated the role of fire regime (fire frequency and time since last fire) on the regeneration strategies (resprout vs. seedlings) and how it would shape the resilience of tropical savannas. Therefore, we asked: Does the importance of regeneration strategies change in recently burned tropical savannas submitted to long-term fire exclusion with different fire frequencies? We analyzed the importance of regeneration strategies through the potential regeneration (belowground bud bank and seed bank before fire) and the observed regeneration (counting the resprouting and seedlings recruited after fire).

3. Plant community recovery from the belowground bud bank showed to be the most important regeneration strategy in tropical savannas of Cerrado, even after >30 years of fire exclusion. Plant communities under higher fire frequency and lower time since last fire recovered faster than the ones under lower fire frequency and longer periods of fire exclusion. Moreover, regeneration from the seed bank showed lower contribution for post-fire plant community recovery.

4. *Synthesis.* The belowground bud bank is crucial for post-fire regeneration in Tropical savannas even after longer periods of fire exclusion and low fire frequency. Although the seed bank gains more importance, it is not the primary regeneration strategy. Therefore, maintaining a viable belowground bud bank is essential to ensure the resilience of tropical savannas such as the Cerrado.

Introduction

Disturbance events, such as fire, play a crucial role in shaping plant communities by acting as an important ecological filter for plant species to persist in the community, while also selecting plant traits and influencing their regeneration strategies (Grime 1997; Keeley et al. 2011; Pausas & Keeley 2014). Vegetation recovery after fire is an important process in plant community assembly, and thus plant regeneration strategies determine the persistence of plant species in the aboveground plant community (Keeley & Zedler 1978; Pausas et al. 2004; Clarke et al. 2015). Two main mechanisms allow plant species to persist in ecosystems under recurrent fire events: resprouting from bud banks or recruitment of seedlings from the seed bank (Bond & Midgley, 2001; Pausas & Keeley, 2014).

Plant communities in fire-prone ecosystems are adapted to the fire regime that selects plant species to persist, particularly under a given fire frequency (Keeley & Zedler, 1978; Pausas & Keeley, 2014). There are two mechanisms by which plants persist under recurrent disturbances: resprouting and seedling recruitment. Both strategies coexist, resprouting from the bud bank and seed germination from the seed bank, each one prevails in the ecosystem according to the fire regime (Keeley & Zedler, 1978; Pausas & Keeley, 2014). In fire-prone ecosystems subjected to frequent and low intensity fires, resprouting species are usually favored (Keeley & Zedler 1978; Bond & Midgley 2003). Conversely, ecosystems subjected to high intensity and low fire frequency may lead to higher plant mortality, affecting resprouting responses by damaging viable buds. On the other hand, the longer interval between fires favor the fulfillment of the seed bank and as a consequence, the recruitment of seedlings (Keeley & Zedler 1978).

It is well-known that tropical savannas in Brazil, the Cerrado, have evolved in the presence of fire for at least four million years (Simon et al., 2009). The fire regime in the Cerrado is characterized by low intensity and frequent surface fires, with returning intervals ranging from 3 to 5 years (Coutinho, 1982; Miranda, 2009). Fire, by consuming the aboveground biomass, generates gaps that can act as safe sites for seedling recruitment, by increasing sunlight on the

soil surface, changing the microclimate close to the soil (Fidelis & Blanco 2014, Daibes et al. 2017) and diminishing plant competition for space during the seedling establishment (He et al. 2019). In addition, fire selects different functional traits such as germination stimulated by temperature and smoke (e.g. Dairel & Fidelis, 2020a; Keeley et al., 2011; Zirondi et al., 2019), post-fire flowering (e.g. Fidelis & Zirondi, 2021; Zirondi et al., 2021), bud protection (e.g. Charles-Dominique et al., 2015, Chiminazzo et al. 2021), and resprouting capacity from bud-bearing organs (Fidelis et al. 2014, Bombo et al. 2022) that contribute to the resilience and persistence of plants in post-fire environmental conditions.

In general, resprouting is the main post-fire regeneration strategy associated with frequent fires in tropical savannas (Bond & Midgley, 2001; Pilon et al., 2021; Zupo et al., 2021). New resprouts came from both aboveground and belowground buds. To persist after fire, plants that invest in aboveground buds, such as woody species, are dependent on how species can protect their buds (Charles-Dominique et al. 2015; Chiminazzo et al. 2021). On the other hand, many species in the herbaceous layer develops buds on belowground structures, such as xylopodia, bulbs and rhizomes (Pausas et al. 2018; Bombo et al. 2021; Bombo et al. 2022) which avoid damage from the high temperature and from the flames enabling these species to persist in post-fire environmental (Pausas et al. 2018).

Nevertheless, long-term fire exclusion might decrease the bud bank density (Fidelis et al. 2014; Bombo et al. 2022), affecting the way plants will regenerate in the post-fire environment. Thus, when intervals between fire events are long and the bud bank size has decreased, seedling recruitment could emerge as a significant strategy following fire events (Coutinho, 1990; Pilon et al., 2021). In this case, where tropical savannas are subjected to long-term fire exclusion and characterized by a history of low-frequency fire events, the importance of resprouting and seedling recruitment strategies in contributing to vegetation recovery may vary significantly. Although efforts have been made to understand the influence of fire regime on post-fire community

dynamics, post-fire seed germination as a regeneration mechanism has shown limited contribution to plant community post-fire regeneration (Pilon et al. 2021; Zupo et al. 2021).

We aimed thus, to investigate how fire frequency and time since last fire would affect regeneration strategies and how it would shape the resilience of tropical savannas. Therefore, we focused on the following question: Does the importance of regeneration strategies change in savannas that have low fire frequency and different times since last fire? We analyzed the importance of regeneration strategies in two-ways: I)-potential regeneration: regeneration source from the belowground bud bank and seed bank before fire and, II)- observed regeneration: strategy recruited by resprouting and seedlings after fire. We hypothesize that in tropical savannas that have been exposed to more fire events and have shorter time since last fire, resprouting from the bud bank would still be the most important regeneration strategy. On the other hand, the longer the fire exclusion and the lower the fire frequency is, the higher the importance of the seed bank and germination as a regeneration strategy. Fire frequency previously observed in study sites would be an important ecological factor influencing the bud bank and seed bank density, affecting the potential regeneration source that the plant community has to recover.

Material and methods

Study site

This study was conducted in southeastern Brazil at the Estação Ecológica de Itirapina (EEI, hereafter; 22° 11' to 22° 15' S, 47° 51' and 48° 00' W, 750m a.s.l), in the Cerrado. The vegetation is a mosaic of savannas and other vegetation types, ranging from open to closed ecosystems (e.g. forest) (Zanchetta et al., 2006). The study was carried out in an area of open savannas (*campo sujo*), characterized by a continuous species-rich herbaceous layer, composed mainly by C4 grasses and forbs, with few scattered shrubs and dwarf trees (Coutinho, 1978). The climate in the region is seasonal, with well-marked rainy summers (October-March) and dry

winters (April-September), with mean annual precipitation ca. 1400 mm/year. Mean annual temperature ranges from 17 °C and 24°C in winter and summer seasons, respectively (Zanchetta et al., 2006). Soils in the study site are predominant oxisols and entisols with high sand content (Zanchetta et al., 2006).

The EEI became a protected area during the 1980s (Zanchetta et al., 2006) and as a result, fire was suppressed due to the fire-zero policy at that time (Durigan & Ratter, 2016). Despite the fire suppression policies, about 3,5% of the native vegetation at the EEI was submitted to fire events in the last 33 years, creating a mosaic with patches with different fire frequencies and time since last fire (Conciani et al., 2021). To assess the relationship between fire regime and regeneration strategies, we selected two sites at the EEI with different number of fire events and time since last fire since 1985: FE1985 – 35 years of fire exclusion, with one fire event in 1985, and FE2009 – 12 years of fire exclusion, with three fire events, being the last one in 2009 (Conciani et al., 2021).

Methods

In each site, FE1985 and FE2009, we established four experimental plots (50 x 50 m) which underwent prescribed fires in the dry season of 2020 (August), (four plots per site x two sites = 8 plots). To avoid pseudoreplication, the experimental fires were conducted individually. To replicate natural fire conditions, the prescribed fire was set in the wind direction (head fire). The prescribed fires were carried out by the EEI fire brigade. Additionally, firebreaks (five meters wide) were implemented around each plot before the prescribed fires.

Data sampling was performed before and after the prescribed fires to assess the importance of regeneration strategies in tropical savannas with distinct fire regimes. The sampling prior to the fire was to assess the potential regeneration (bud bank and seed bank) of the ecosystem. The sampling after fire reintroduction was to assess the observed regeneration (ramets and seedlings) post-fire.

Belowground bud bank and seed bank sampling (potential regeneration)

To sample the belowground bud bank, we randomly established eight subplots (0.25 m²) within each experimental plot in sites FE1985 and FE2009 (4 experimental plots x 8 subplots of bud bank samples x 2 sites = 64 sampling units). In each sampling unit, the aboveground biomass was clipped close to the soil surface. Further, the soil was excavated using a shovel (0.5 x 0.5 x 0.1m), because the belowground buds from the herbaceous layer are concentrated at depths ranging from 0 to 5 cm (Klimešová et al. 2019; Bombo et al. 2021; Bombo et al. 2022). The soil samples were sieved in the field, and all plant structures were separated, stored in plastic bags, with respective codes, and brought to the lab to be stored at -18°C before analysis. The material was later defrosted in the lab and plant structures were sorted according to their belowground organ types (organs diversity) (Pausas et al. 2018: fleshy rhizomes, stems, gemmiferous roots, woody rhizomes and xylopodia) and according to their growth forms (growth form diversity): graminoid, forb and shrub. Viable buds were counted using a stereomicroscope. The number of buds in all organs were counted and the bud bank was quantified in terms of buds per m².

To sample the soil seed bank, we randomly collected five soil cores (0.2 x 0.2 x 0.02m) within each experimental plot in each site, FE 1985 and FE2009 (4 experimental plots x 5 soil cores x 2 sites = 40 soil seed bank cores). Soil samples were sieved to eliminate plant structures and stones (Heerd et al., 1996). Afterwards, soil samples were transferred into aluminum trays (27.5 x 18.5 x 4.5 cm), containing holes at the bottom of each tray to prevent water accumulation, and allocated in a greenhouse irrigated three times a day (mean of 36 ml of water/day). Using the seedling emergence method (Heerd et al., 1996), we estimated seed density (seeds/m²) making observations of seedling emergence every 15 days for four months. After seed germination, most of the seedlings were identified at the species level (more details see Dairel & Fidelis, *submitted*) and classified according to their growth form: graminoid, forb and shrub.

Post-fire regeneration: number of resprouts vs seedlings (observed regeneration)

To assess the regeneration strategy of plants after fire (resprouting x germination), we randomly established 10 subplots (0.25 m^2) in three experimental plots in each site, FE1985 and FE2009 (3 experimental plots x 10 subplots for counting strategies x 2 sites = 60 sampling units). The subplots were sampled in the first rainy season (February 2021, 6 months post-fire) after fire. In each subplot, we recorded the number of resprouts (ramets) and seedlings. The resprouts were identified as all new shoots coming from the base and belowground, while seedlings were identified by the presence of the cotyledons. We measured the total number of new resprouts (ramets) and seedlings per m^2 .

Data Analysis

To test the differences between bud and seed bank density (potential regeneration), as well as the number of resprouts and seedlings (observed regeneration) in areas with different fire frequencies and time since last fire, we performed a generalized linear mixed models (GLMM, fixed factor: site, random effect: plot, family: Poisson, Zuur et al., 2009) using the *glmer* function from the *lme4* package in R. When response variables (bud bank density, seed bank density, resprouts and seedlings counting) were not normally distributed, we applied a $\log_{10}(n + 1)$ transformation. We compared sites, FE1985 and FE2009, for the potential regeneration, using the total number of buds. m^2 and the total number of seedlings. m^2 . Furthermore, we compared sites FE1985 and FE2009, for the observed regeneration using the number of seedlings and resprouts (ramets) per m^2 after fire. To compare bud and seedlings proportion per growth form (graminoid, forb and shrub) between sites (FE1985 and FE2009), we performed permutational multivariate variance analysis (PERMANOVA) using the *adonis* function from the *vegan* package (Oksanen et al., 2020). We carried out the PERMANOVA with 1000 permutations using the *strata* function to limit permutations only between sites (FE1985 and FE2009). Analyses were performed

using the R version 4.2.2 and we used the *ggplot 2* package (Wickham, 2016) and *wesanderson* package (Ram et al., 2023) for visualization graphics.

Results

Resprouting was the dominant strategy in both FE1985 and FE2009 sites (Fig1). Seedling recruitment, on the other hand, represented only 5% in both sites. The bud bank had higher density of propagules compared to the seed bank, for both sites (Fig.1).

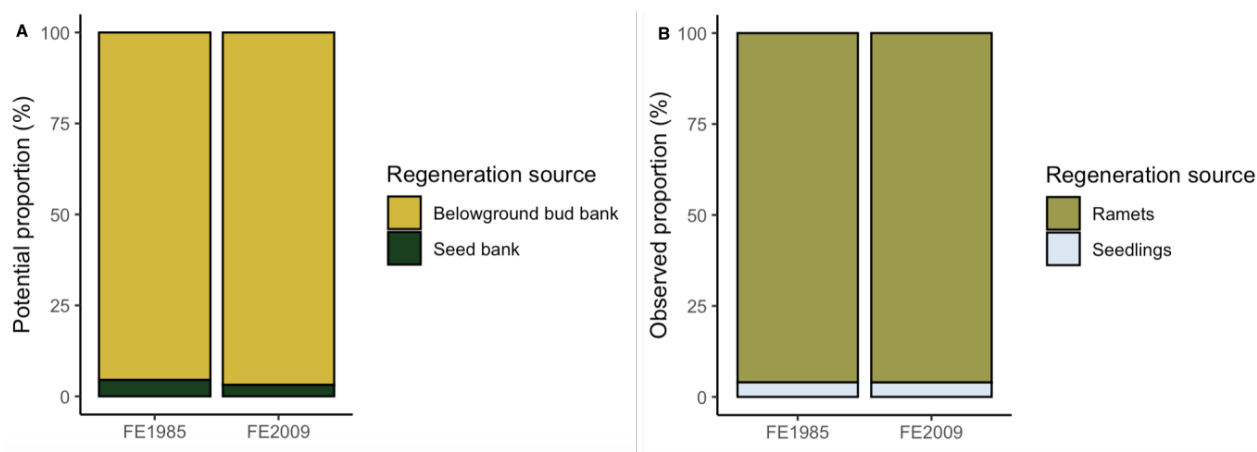


Figure 1. Proportion (%) of potential regeneration (A) and observed regeneration (B) source by belowground bud bank and seed bank in fire-excluded tropical savannas with different fire regime: FE1985– 33 years of fire exclusion, with one fire event in 1985, and FE2009 – 12 years of fire exclusion, with three fire events since 1985, being the last one in 2009, in the southeastern Brazil at Estação Ecológica de Itirapina.

Both FE1985 (1293.05 ± 155.34 buds.m²) and FE2009 sites (1171.6 ± 175 buds.m²) had similar potential to resprout according to their bud bank density (Fig.2A; Supplementary information – Table S1). About 40% of belowground bud banks were composed of graminoid buds for FE1985 and FE2009 (Fig. 2B). Despite the bud proportion per growth form not being statistically different between sites, we found a lower proportion of forb buds (18%, Fig. 2B) and

a greater proportion of shrub buds (40%, Fig. 2B) at site FE1985 in comparison to FE2009 (28% and 30%, for forb and shrub buds, respectively, Fig. 2B).

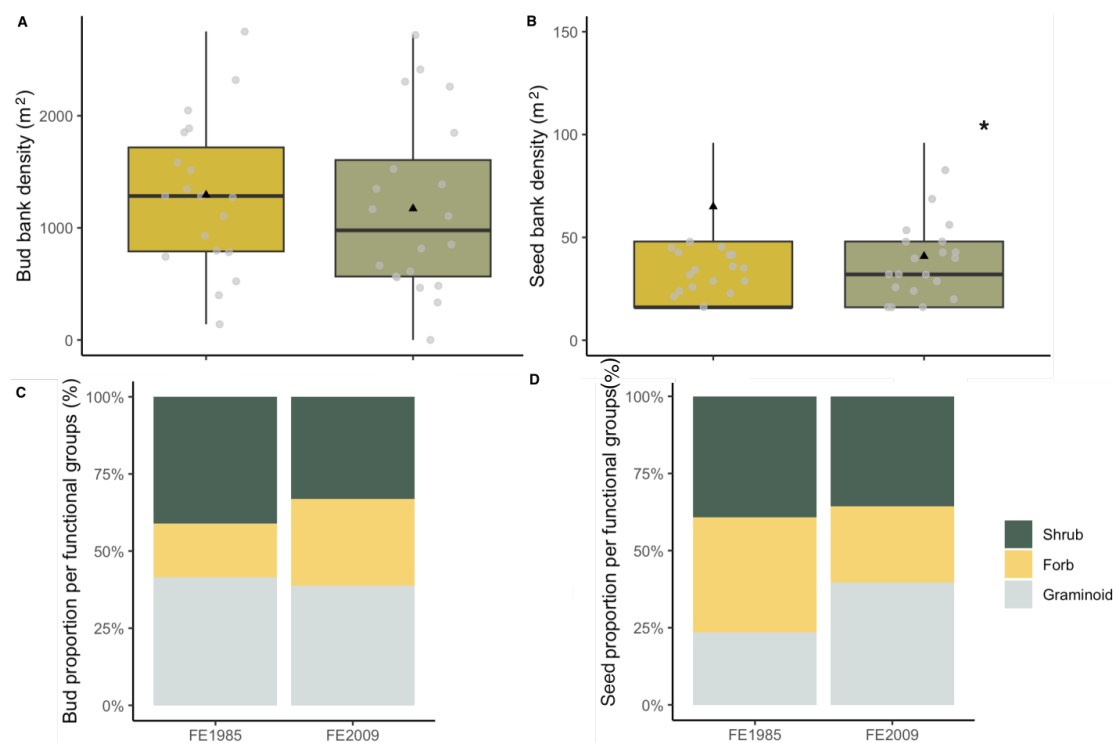


Figure 2. Bud bank density (buds.m², A) and bud proportion (%) per growth forms (graminoid, forb and shrub, B), as well as seed bank density (seedlings.m², C) and seed proportion (% , D) per growth forms in fire-excluded tropical savannas with different fire regime: FE1985– 33 years of fire exclusion, with one fire event in 1985, and FE2009 – 12 years of fire exclusion, with three fire events since 1985, being the last one in 2009, in the southeastern Brazil at Estação Ecológica de Itirapina. (A–C) Triangle markers indicate mean of belowground bud bank and seed bank density, respectively. Asterisk (*) indicates significant difference between sites ($p < 0.001$; for GLMM models with fixed factor: site, random effect: plot, family: Poisson).

Site FE1985 (64.9 ± 16.4 seedlings.m², Fig. 2C) presented a higher density of seeds stored in the soil seed bank compared to FE2009 ($p < 0.001$, 40.8 ± 4.8 seedlings.m², Fig. 2C, Supplementary information – Table S2). At FE1985, shrubs were the main component in the seed bank, accounting for 47%, followed by forbs (33%) and graminoids (20%, Fig. 2D). On the other

hand, FE2009 had a seed bank dominated by graminoids (42%), followed by forbs (40%) and shrubs (18%, Fig. 2D).

The belowground organ density did not differ between FE1985 (140 ± 13 belowground organs.m²) and FE2009 (129 ± 15 belowground organs.m², Supplementary information–Fig.S1, Table S3). The belowground community was mainly dominated by rhizomatous structures, both fleshy and woody rhizomes (Supplementary information–Fig.S1), which together accounted for 80% of the community. Moreover, the proportion of the different types of belowground organ did not differ between sites FE1985 and FE2009 (Supplementary information–Fig.S1B).

A higher number of ramets was found at FE2009 (88.1 ± 14.9 ramets.m², Fig. 3A, $p=0.01$) compared to FE1985 (43.87 ± 5.41 ramets.m², Fig. 3A; Supplementary information – Table S4). Despite the high potential regeneration from the belowground bud bank (Fig. 2A), only 3% of the potential viable buds turned into ramets after fire at FE1985, and 8% was recruited at FE2009. The number of new ramets resprouted was significantly different among growth forms in both sites FE1985 (Fig.3B; $p<0.01$) and FE2009 (Fig.3B; Supplementary information – Table S4).

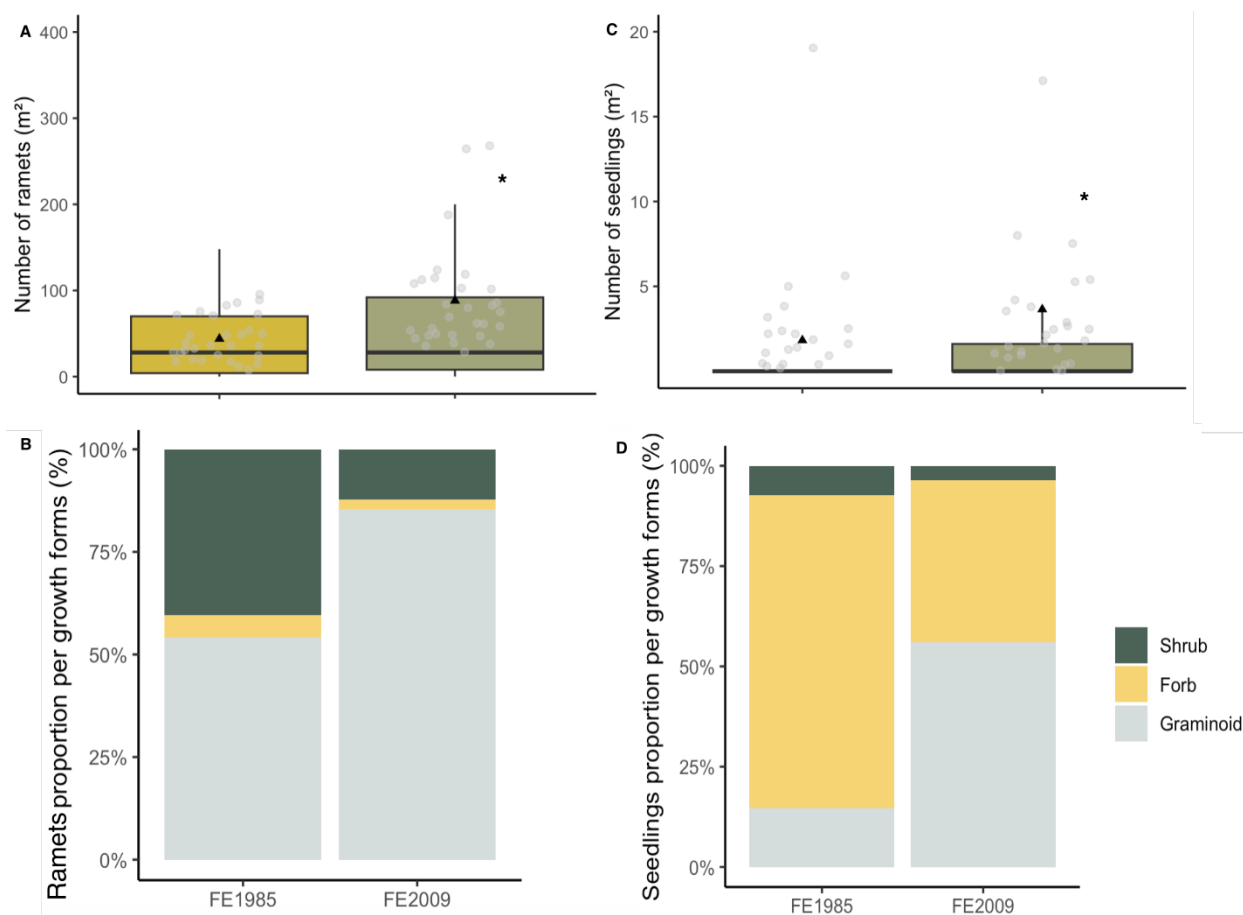


Figure 3. Number of ramets (ramets.m², A) and ramets proportion (% , B) per growth forms (graminoid, forb and shrub, B), as well as number of seedlings (seedlings.m², C) seedlings proportion per growth form (% , D) recruited after fire in fire-excluded tropical savannas with different fire regime: FE1985– 33 years of fire exclusion, with one fire event in 1985, and FE2009 – 12 years of fire exclusion, with three fire events since 1985, being the last one in 2009, in the southeastern Brazil at Estação Ecológica de Itirapina. Triangle markers indicate mean of seedlings. Asterisk (*) indicate statistical difference among sites ($p=0.04$; for GLMM models with fixed factor: site, random effect: plot, family: Poisson).

Seedling recruitment at FE2009 was higher compared to FE1985 ($p=0.04$, FE1985= 1.82 ± 0.57 seedlings.m² and FE2009= 3.64 ± 1.06 seedlings.m², Fig. 3C; Supplementary information – Table S5). Only 3% of the seed bank potential was effectively recruited at FE1985, while 9% was recruited at FE2009. Furthermore, the number of seedlings recruited from the seed

bank is significantly different among growth form in both site FE1985 (Fig. 3D; $p < 0.01$) and FE2009 (Fig. 3D; Supplementary information – Table S5).

Discussion

Plant regeneration from the bud bank showed to be the most important regeneration strategy in our study site, a tropical savanna from the Cerrado, even after more than 30 years of fire exclusion. Despite the different fire regimes, the belowground bud bank in terms of total buds and per growth form is similar between sites (FE1985 and FE2009), with a higher number of ramets resprouting in FE2009 post-fire. Seed bank recruitment contributed less for post-fire plant community recovery for both sites. FE1985 presented higher seed density in the seed bank, however, the number of seedlings recruited at FE2009 was higher compared to FE1985.

Regardless of the distinct fire frequencies and time since last fire from both sites, we observed no decrease in the bud bank density. The bud bank still presented a high number of buds, however, there were no differences in bud bank diversity per growth form between sites. We observed that with longer time since last fire, the belowground bud bank of forbs decreases while shrub bud density increases. Conversely, for the seed bank, we observed the opposite trend: higher forb seeds density, with a lower graminoid seed density at FE1985. These results show us that growth forms will regenerate from different sources according to time since last fire: graminoid species will rely mostly on the belowground bud bank for post-fire regeneration in sites where fire has been excluded for shorter time, while while forbs will be dependent on seedling recruitment from the seed bank, since a drastic decrease in the forb bud bank can be observed when fire is excluded (Bombo et al. 2022). We should point out that, although forb bud bank showed a decrease in number of buds, they could still regenerate from the seed bank, while graminoids would have a lower success germinating after fire due to the lower seed density.

Forb species, an important component of the species-rich herbaceous layer (REF), are being highly threatened by fire exclusion (Bombo et al. 2022). Plant communities with frequent

fires showed high diversity of belowground bud bank, highlighting the presence of xylopodia - organ type associated with forbs and shrubs species, and frequent fires (Fidelis et al. 2014, Bombo et al. 2022). As a consequence, recruitment of new forb ramets after fire is compromised, affecting the herbaceous layer composition and resilience, once forb regeneration will be more dependent on seedling recruitment.

The belowground bud bank had a high percentage of graminoids buds and its species showed to rely on this regeneration source since we had a high graminoid ramet recruitment for both sites, FE1985 and FE2009. Graminoids are the most resilient component in tropical savannas (Bond, 2004), being able to rapidly resprout after fire from basal buds which are located close to the soil surface protected by the plant architecture (Pilon et al. 2021, Rachid 1956). Graminoids are mainly caespitose species composed of old leaf sheaths that stay together in the plant individual protecting their buds, which allows grasses to regrow after disturbances (Zimmermann et al., 2010). Despite the importance of the belowground bud bank for post-fire regeneration, we observed a high percentage of seedling recruitment of graminoids species, mainly attributed to a perennial species (*Sporobolus aeneus* (Tring.) Kunth) that produces a substantial quantity of seeds with high viability (Dairel & Fidelis, 2020a), notably enhancing graminoid seedling recruitment at site FE2009 (also see Dairel & Fidelis, submitted). However, at FE1985 site, the contribution of graminoids to the seed bank is very low, which can be correlated with the low production of viable seeds (Dairel & Fidelis, 2020a; Fontenele et al., 2020), forming thus transient seed banks (Aires et al., 2014; Dairel & Fidelis, 2020b) that remain viable for about six months (Dairel & Fidelis, 2020a) up to one year, influencing the success of graminoids seedling recruitment.

FE1985 presented a higher percentage of buds of shrub and seeds of shrub in both the belowground bud bank and seed bank. The low recruitment of shrub species after fire can be attributed to distinct factors, such as the presence of dormant seeds in which direct (heat-shock, smoke) and indirect (temperature fluctuation) effects of fire were not enough to trigger their

germination from the seed bank. However, at FE1985 shrub species had low seedling recruitment after fire probably because seeds did not survive the fire passage, showing a shift from fire-resistant seed species to fire-sensitive ones (Dairel & Fidelis, submitted). In these areas, only few shrub species are recruited from seeds, such as *Baccharis dracunculifolia* DC., a seeder species (Galíndez et al., 2009, Dairel & Fidelis, submitted). This suggests that *Baccharis dracunculifolia* may not have a niche compatible with post-fire conditions, potentially making it a fire-sensitive species. Additionally, at FE2009, the percentage of shrub propagules was higher in the bud bank than in the seed bank, indicating that the recruitment from the bud bank increases with shorter time since last fire.

The comparison of the regeneration strategies (observed recruitment by new ramets and seedlings) revealed that site FE2009 had higher propagules to regeneration after fire by both strategies. In general, the belowground bud bank plays an important role on post-fire regeneration in savannas from the Cerrado, even after longer periods of fire exclusion and low fire frequency. However, the seed bank as a source of propagules for post-fire regeneration showed to gain importance in tropical savannas, mostly for forbs. Despite that, the seed bank was not the main regeneration strategies for tropical savannas, as well as post-fire seed germination. Therefore, to maintain tropical savannas resilience is of high importance the maintenance of a viable belowground bud bank. The changes observed in the belowground community may be irreversible and thus, affecting the resilience of tropical savannas as the Cerrado.

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How does fire regime shape regenerations strategies in tropical savannas?

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Supplementary information

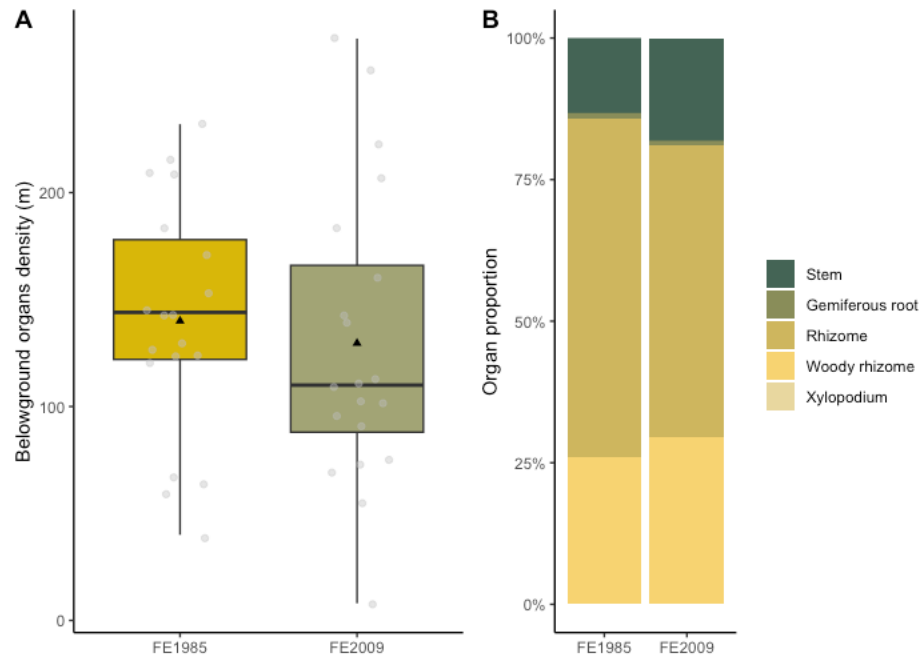


Figure S1. Belowground organs density (organs.m², A) and organ proportion (% , B) per functional groups (graminoid, forb and shrub) in fire-excluded tropical savannas with different fire regime: FE1985– 33 years of fire exclusion, with one fire event in 1985, and FE2009 – 12 years of fire exclusion, with three fire events since 1985, being the last one in 2009, in the southeastern Brazil at Estação Ecológica de Itirapina. (A) Triangles forms indicates mean of belowground organs density.

Table S1. Total belowground bud bank density (number of buds/m²; mean $\pm$ SE) and bud bank density according to growth forms (graminoid, forb and shrub) in fire-excluded tropical savannas with different fire regime: FEFE1985– 33 years of fire exclusion, with one fire event in 1985, and FE2009 – 12 years of fire exclusion, with three fire events since 1985, being the last one in 2009, in the southeastern Brazil at Estação Ecológica de Itirapina.

Fire regime	Total	Graminoid	Forb	Shrub
FE1985	1293 $\pm$ 155.3	510 $\pm$ 144	226 $\pm$ 44.1	534. $\pm$ 27.8
FE2009	1171.6 $\pm$ 175.1	453 $\pm$ 1.71	333 $\pm$ 41.6	388 $\pm$ 18.5

Table S2. Total seeds density (m², mean $\pm$ SE) and seed density according to growth forms (graminoid, forb and shrub) in fire-excluded tropical savannas with different fire regime: FEFE1985– 33 years of fire exclusion, with one fire event in 1985, and FE2009 – 12 years of fire exclusion, with three fire events since 1985, being the last one in 2009, in the southeastern Brazil at Estação Ecológica de Itirapina.

Fire regime	Total	Graminoid	Forb	Shrub
FE1985	64.9 $\pm$ 16.4	14.1 $\pm$ 3.6	22.7 $\pm$ 9.8	33.1 $\pm$ 15.2
FE2009	40.8 $\pm$ 4.8	13.7 $\pm$ 1.8	14.6 $\pm$ 2.7	6.1 $\pm$ 0.7

Table S3. Total belowground organs density (number of organs/m²; mean $\pm$ SE) and belowground density according to organs diversity (stem, gemmiferous root, rhizome, woody rhizome, xylopodia) in fire-excluded tropical savannas with different fire regime: FEFE1985– 33 years of fire exclusion, with one fire event in 1985, and FE2009 – 12 years of fire exclusion, with three fire events since 1985, being the last one in 2009, in the southeastern Brazil at Estação Ecológica de Itirapina.

Fire regime	Total	Stem	Gemmiferous root	Rhizome	Woody rhizome	Xylopodia
FE1985	140 $\pm$ 12.7	28.8 $\pm$ 1.1	24.32 $\pm$ 0.7	42.8 $\pm$ 0.7	43.4 $\pm$ 5.3	1 $\pm$ 1
FE2009	129.6 $\pm$ 14.5	36.8 $\pm$ 1.5	18.8 $\pm$ 0.9	39.3 $\pm$ 0.9	36.9 $\pm$ 3.4	0

Table S4. Number of ramets recruitment (m^2 , mean $\pm$ SE) according to growth forms (graminoid, forb and shrub) in fire-excluded tropical savannas with different fire regime: FEFE1985– 33 years of fire exclusion, with one fire event in 1985, and FE2009 – 12 years of fire exclusion, with three fire events since 1985, being the last one in 2009, in the southeastern Brazil at Estação Ecológica de Itirapina.

Fire regime	Total	Graminoid	Forb	Shrub
FE1985	43.8 $\pm$ 5.4	23.7 $\pm$ 3.7	2.2 $\pm$ 0.62	17.68 $\pm$ 2.4
FE2009	88.1 $\pm$ 14.8	75.2 $\pm$ 10.7	2.1 $\pm$ 0.43	10.8 $\pm$ 1.2

Table S5. Number of seedlings recruitment (m^2 , mean $\pm$ SE) according to growth forms (graminoid, forb and shrub) in fire-excluded tropical savannas with different fire regime: FEFE1985– 33 years of fire exclusion, with one fire event in 1985, and FE2009 – 12 years of fire exclusion, with three fire events since 1985, being the last one in 2009, in the southeastern Brazil at Estação Ecológica de Itirapina.

Fire regime	Total	Graminoid	Forb	Shrub
FE1985	1.8 $\pm$ 0.6	0.27 $\pm$ 0.1	2.2 $\pm$ 0.62	0.33 $\pm$ 0.1
FE2009	3.6 $\pm$ 1.1	2.1 $\pm$ 0.9	1.5 $\pm$ 0.3	0.13 $\pm$ 0.1

Fire stimulates seedling recruitment from the seed bank in the Cerrado

Running title: fire effects on seed bank recruitment in Cerrado

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16

Abstract

1. **Questions.** Fire is an important ecological factor influencing plant communities in many fire-prone ecosystems. Savannas in the Cerrado are resilient and fire, with plants exhibiting fire-related traits, allowing them to persist in post-fire environments. Therefore, excluding fire results in changes in plant community dynamics, thus affecting their resilience. We investigated the effect of the reintroduction of fire in savannas where fire has been excluded for longer periods (>12 years) on seedling recruitment. We asked the following questions: I) How does fire affect seed bank germination in sites with different fire histories and II) How did fire exclusion affect species composition of the seed bank?
2. **Location.** The study was carried out at the Estação Ecológica de Itirapina (EEI) Southeastern Brazil.
3. **Methods.** Two sites with different fire histories were selected: FE1985 – fire exclusion >30 years, with one fire event in 1985, and FE2009 – fire exclusion for 12 years, with three fire events since 1985, being the last one in 2009. Both areas have low fire frequency, but different times since the last fire. Soil samples were collected before and after prescribed fires to evaluate the effects of fire after longer periods of fire exclusion in the soil seed bank. Using the seedling emergence method, we evaluate the effects of the reintroduction of fire after longer periods of fire exclusion in the soil seed bank.
4. **Results.** We found that fire significantly increased the seed bank recruitment at both sites, FE1985 and FE2009 (increase of 16% and 50% in seedling recruitment, respectively), showing that species of the Cerrado responded positively to the fire passage. The reintroduction of fire promoted different effects on seed bank recruitment: shrubs experienced a significant decrease in seedling emergence from the seed bank at FE1985, whilst their recruitment was not affected at FE2009. Time since last fire appears to influence the seed bank

composition, showing a shift in dominance from a grassy community to a woody one.

5. **Conclusions.** After fire, more species germinated from the seed bank showing that direct and indirect effects of fire are affecting seed germination from the seed bank, showing its importance on seedling recruitment from the seed bank in the Cerrado. .

Keywords: fire, seedling recruitment, seed bank, Tropical savannas, post-fire regeneration, fire-related germination cues

1. Introduction

In fire-prone ecosystems, factors like resource availability, system productivity and fire regime, mainly fire frequency (Bond & Van Wilgen 1996; Keeley et al. 2011), significantly influence post-fire plant community regeneration (Bond & Van Wilgen 1996; Bond & Keeley 2005). These communities present a set of functional traits that allow them to be highly resilient to fire events, both resprouting from protected buds (Clarke et al. 2013) and stimulating seedling recruitment are considered an important regeneration mechanism (Moreira et al. 2010). These responses may vary according to fire intensity and frequency: frequent and low intensity fires (e.g. tropical savannas) favor resprouting species (Keeley & Zedler 1978; Bond & Midgley 2003) which rely on a viable bud bank as regeneration source (Pausas et al. 2018, Bombo et al. 2022). On the other hand, systems with low-frequent, but high-intensity fires seeder species (Keeley & Zedler 1978) with the seed bank playing a central role as the source of propagules for vegetation regeneration, as it occurs in Mediterranean vegetation (Pausas & Keeley 2014). The source of propagules, either from the seed bank or the bud bank, is an essential component of plant community, having a key role in the post-disturbance regeneration, resilience and on the maintenance of the post-fire plant community stability (Kiss et al. 2018; Török et al. 2020).

Tropical grassy biomes cover 20% of global ecosystems (Parr et al. 2014) and fire is the main disturbance maintaining their structure, function and diversity (Bond & Keeley 2005; Bowman et al. 2009). In the Cerrado, plants present a set of fire-related functional traits and strategies, assuring their resilience capacity, such as resprouting from protected buds (Chiminazzo et al. 2021; Pilon et al. 2021; Zupo et al. 2021), stimulated seed germination by heat-shock and smoke (Zirondi et al. 2019; Dairel & Fidelis 2020a; Zupo et al. 2021) and enhanced post-fire flowering (Pilon et al. 2018; Zirondi et al. 2021; Fidelis et al. 2022).

Resprouting is the most important regeneration strategy for plants in the herbaceous layer of the Cerrado (Pilon et al. 2021; Zupo et al. 2021). Despite the

major role played by the bud bank in post-fire ecosystems (Fidelis et al. 2014; Bombo et al. 2022), plant species can persist and regenerate by recruiting from seeds stored in the seed bank (Thompson & Grime 1979; Dairel & Fidelis 2020b). Overall, resprouting is the post-fire strategy most linked with frequent fires, however, long-term fire exclusion may lead to a decrease in the bud bank resources (Fidelis et al. 2014; Bombo et al. 2022). In this scenario of fire exclusion and lower bud bank, the seed bank can emerge as a potential resource of regeneration if fire occurs or is reintroduced in the system. Since the following decades of fire suppression in the Cerrado (Durigan & Ratter, 2016), evaluating fire exclusion effects has become an important topic, mostly to support restoration and conservation actions, supporting the reintroduction of fire as a management tool to maintain plant community structure and diversity (Schmidt et al., 2018; Durigan 2020).

Several treatments mimetizing directly and indirectly fire conditions such as daily temperature fluctuations (Daibes et al. 2017; Dairel & Fidelis 2020a), heat-shock and/or smoke (Ramos et al. 2016; Zirondi et al. 2019; Dairel & Fidelis 2020a; Zupo et al. 2021) shows that, despite being resistant, few species of the Cerrado respond to fire-related germination cues. Furthermore, we still lack information about field experiments of fire effects on seedling recruitment from the seed bank in the Cerrado, since most germination experiments are conducted in the laboratory, under controlled conditions to simulate the passage of fire on seed germination through heat-shock, smoke, temperature fluctuation on the soil surface or even as their combination.

To understand the role of fire on the seedling recruitment from the seed bank in areas where fire was excluded for longer periods (>12 years), we investigated the effects of fire on the seed bank. We chose these areas because they had a low fire frequency (1 and 3 fire events), but different times since last fire, which may have influenced the role of the seed bank in the post-fire regeneration of the vegetation (Keeley & Zedler 1978). Therefore, we focused on two questions: I) How does fire

affect seed bank germination in sites with different fire histories and II) How did fire exclusion affect species composition of the seed bank? We hypothesize that the reintroduction of fire after long-term fire exclusion will influence the seedling recruitment from the seed bank, with more species germinating after fire, showing that the direct (e.g. heat shock, smoke, ashes) and indirect effects (e.g. temperature fluctuations, light) of fire will have a positive effect on seed germination from the seed bank. In addition, different fire histories will influence the seed bank composition promoting different responses in post-fire seedling recruitment, such as triggering seedling recruitment or being lethal for the seeds stored in the seed bank.

2. Methods

2.1. Study area

The study was carried out at the Estação Ecológica de Itirapina (EEI, Southeastern Brazil, 22° 11' to 22° 15' S, 47° 51' and 48° 00' W). Located in the Cerrado, the EEI has a total area of 2,300 ha, comprehending different vegetation types, ranging from open ecosystems (open savannas called *campo sujo*, and wet grasslands) to woodlands and forest (cerrado *sensu stricto*, cerradão, Zanchetta et al. 2006). The climate is classified as Köppen Cwa-type, with a well-marked dry winter from April to September and rainy summers from October to March. Annual mean precipitation and temperature is 1,459 mm and 21,9°C, respectively. In EEI, soils are predominantly sandy Arenosol (Zanchetta et al. 2006).

The study sites were selected based in four criteria: I) open savannas, characterized by a continuous herbaceous layer (composed mostly of C4 grasses and forbs) with scattered shrubs and small trees, II) absence of cattle foraging, III) aboveground vegetation community composed mostly of native species, with maximum of 20% of invasive species cover and, IV) fire-excluded sites with different fire histories and low fire frequency. As a result, two study sites were selected: FE1985 – fire

exclusion for more than 30 years, with one fire event in 1985, and FE2009 – fire exclusion for 12 years, with three fire events since 1985, being the last one in 2009 (Conciani et al. 2021). Both areas have low fire frequency (1-3 fires since 1985), but different times since last fire (fire history, Fig1. A and B).



Figure 1. Fire reintroduction and seed bank recruitment from sites with different fire histories: FE1985 – fire exclusion for more than 30 years (A,C,E), and FE2009 – fire exclusion for 12 years (B,D,F). Sites before (A and B) and during prescribed fires (C and D). Seed bank recruitment after prescribed fires in open savannas of the Cerrado in Southeastern Brazil (E and F).

2.2. Experimental design and data sampling

To evaluate the effects of the reintroduction of fire after longer periods of fire exclusion in the soil seed bank in both fire-excluded sites (FE1985 and FE2009), four fixed plots (50 x 50 m) were established in each site, and the experimental plots were submitted to prescribed fires in August 2020 (dry season, four plots x two sites = eight plots). The experimental fires were conducted individually to avoid pseudoreplication, and they were set in the wind direction (head fire) in order to simulate natural fire

conditions, being all set by the brigade of the EEI (Fig.1 C and D). In addition, firebreaks (five meters wide) were established around each plot before the prescribed fires. We measured fire parameters during prescribed fires. Fires had variable maximum temperatures ranging from 790 to 735°C at sites FE1985 and FE2009, respectively, and residence times ($T > 60^{\circ}\text{C}$) of the fire ranging from 2 to 12 minutes: at FE1985 the residence time ranged from 2 to 6 minutes, while at FE2009, it was from 3 to 12 minutes (personal data).

Soil samples were collected twice (hereafter “treatments”): I) before prescribed fires (one day before fire, BF) and II) after the prescribed fires (AF, Fig.1 E and F). Soil samples were collected three days after fires, since fire induces changes in nutrient availability (Johnstone et al. 2016), as well as nutrient use and acquisition through redeposited ashes (Pellegrini & Jackson 2020). Indirect fire effects, such as daily temperature fluctuation and light conditions in the soil surface are also affected (Keeley et al. 1985; Daibes et al. 2017). In each plot, we collected five soil samples in each treatment per site (five soil samples x four plots x two treatments x two sites = total of 80 soil samples). Considering that in open savannas most seeds in the seed bank are located from 0.5 to 2 cm of the soil layer (Andrade et al. 2002), soil cores were 20 x 20 x 2 cm, including litter, totalizing 800 cm³/sample.

Soil samples were sieved to eliminate stones and plant fragments such as stems and roots, respectively. Samples were transferred into aluminum trays (27.5 x 18.5 x 4.5 cm), containing holes at the bottom of each tray to prevent water accumulation. Using the seedling emergence method (Thompson & Grime 1979), we estimated seed density (m²) and species diversity and composition present in the seed bank of both sites (FE1985 and FE2009) and treatments (before and after prescribed fire). The seed bank density was measured as the total number of seedlings emerged per m². Diversity was measured as the total number of species that emerged. We also classified species into eudicot and monocot and, as well as into different growth forms (graminoids, forb and shrub). Some additional trays filled with sterilized sand were used to evaluate if

there was exogenous contamination. Trays were conditioned in a greenhouse irrigated three times a day (mean of 36 ml of water/day) and observations of seedling emergence were carried out every 15 days for four months. Furthermore, at each observation, the trays were randomly moved around, so they would all be receiving different light and water conditions of the greenhouse. After germination, most of the seedlings were identified at species level (see Appendix S1). Seedlings identified at least at genus level died before we could identify at the species level.

2.3. Data analysis

We tested the effects of the reintroduction of fire on the seed bank density and species richness. To test the effect of the reintroduction of fire on the seed bank density (total, eudicot/monocot, and growth forms), we compared the total number of seedlings emerged between treatments (BF and AF) using a Poisson Generalized Linear Mixed Model (GLMM), with treatments as fixed factor and subplots (where soil samples were collected) as a random effect, for each site separately (FE1985 and FE2009). Since the response variable (seedlings density) did not meet the equal variation assumption, we applied a $\log_{10}(x+1)$ transformation. We used *lsmeans* post-hoc tests to compare pairwise factors with HSD Tukey. Additionally, we carried out a Principal Coordinates Analysis (PCoA) using Bray-Curtis dissimilarity to obtain distance matrices between seed bank composition of both sites (FE1985 and FE2009) and treatments (BF and AF). The effects of fire reintroduction on the seed bank diversity and composition were tested using a permutation multivariate analysis of variance (PERMANOVA) with 10000 permutations. We compared the seed bank diversity and composition between treatments (BF and AF) in both sites (FE1985 and FE2009) using pairwise post-hoc tests of HSD Tukey, including Bonferroni corrections. Furthermore, to analyze the similarity in the seed bank diversity within sites (FE1985 and FE2009) between treatments (BF and AF), we performed the Sørense Index (Sørensen 1948). All

statistical analyses were performed using the R Program (R Development Core Team, 2023), with the following packages: *lme4* (Bates et al. 2015), *lsmeans* (Lenth 2016), *pairwiseAdonis* (Martinez Arbizu, 2017) and *vegan* (Oksanen et al. 2019).

3. Results

Seed bank density

A total of 850 seedlings emerged from FE1985 and 675 seedlings from FE2009 for both treatments, before (BF) and after prescribed fire (AF). At FE1985, mean seed bank density in BF (64.9 ± 16.4 seedlings/m²) did not differ from the density found in AF (68.4 ± 9.7 seedlings/m², $p=0.29$, Fig. 2). On the other hand, at FE2009, mean seed bank density in AF (80 ± 12.2 seedlings/m²; Fig.2) was two-fold the one found in BF (40.8 ± 4.8 seedlings/m²; Fig.2, $p<0.001$). In general, only in FE2009 the reintroduction of fire significantly increased the emergence of seedlings from the seed bank (increase of 50%, $p<0.0001$, Fig.2).

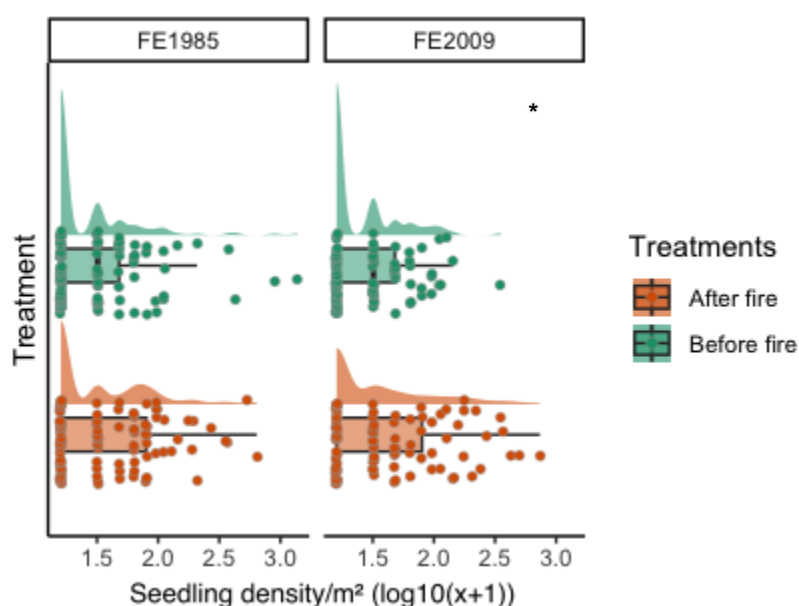


Figure 2. Seed bank seedling density (m^2 , mean $\pm$ SE) from sites with different fire histories: FE1985 – fire exclusion for more than 30 years, and FE2009 – fire exclusion for 12 years, before (BF) and after prescribed fires (AF) in open savannas of the Cerrado in Southeastern Brazil.

The seed bank was predominantly composed of eudicot species in both sites and treatments (FE1985: BF-67%, AF-60%; FE2009: BF-47%, AF-44%; Fig.3). At FE1985, there was no statistical difference of eudicot seedling recruitment between treatments (Fig.3, Appendix S2). On the other hand, fire significantly stimulated the recruitment of eudicot species at FE2009 (Fig.3, see Appendix S2). Finally, monocot species germinated more after fires in both sites ($p < 0.001$, Fig. 3, Appendix S2).

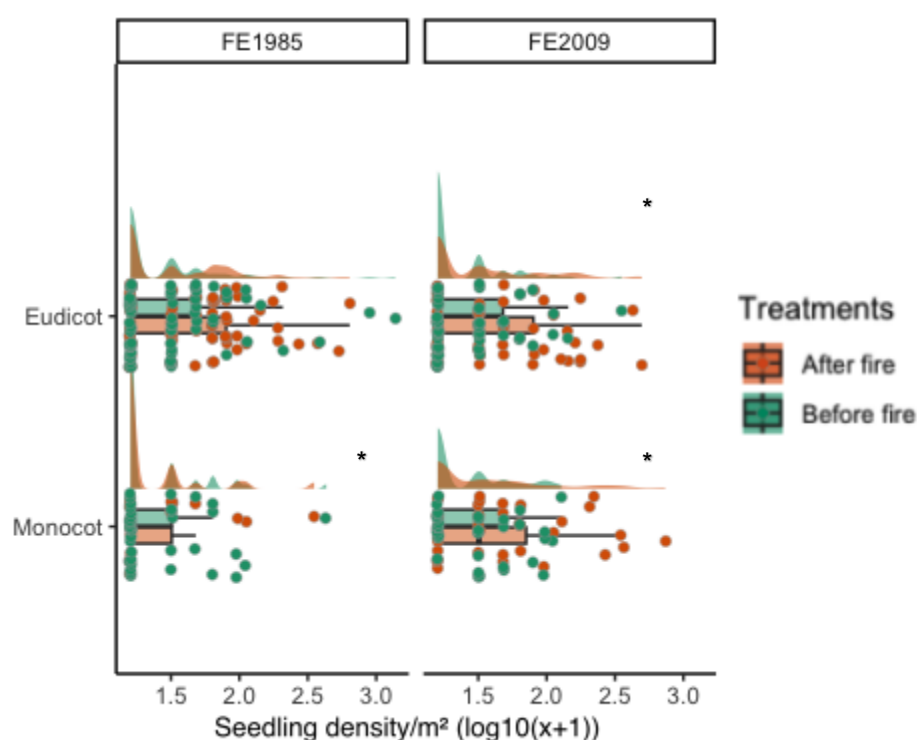


Figure 3. Seedling density (m^2 ; mean $\pm$ SE) of eudicot and monocot species which emerged from the seed bank from sites with different fire histories: FE1985 – fire exclusion for more than 30 years, and FE2009 – fire exclusion for 12 years, before (BF) and after prescribed fires (AF) in open savannas of the Cerrado in Southeastern Brazil.

At FE1985, the seed bank in BF was composed of shrubs (47%), forbs (33%) and graminoids (20%). In this site, fire reintroduction significantly enhanced seedling emergence from the seed bank of forbs and graminoids ($p \leq 0.05$, Fig.4A-B, Appendix S3). However, shrubs had a significant decrease in the seedling emergence from the seed bank after fire (Fig.4C, Appendix S3). At FE2009, the seed bank before fire was dominated by graminoids (42%), followed by forbs (40%) and shrubs (18%). Graminoids and forbs had enhanced seedling emergence from the seed bank after fire (Fig.4C-D), whilst shrub seedling emergence was not affected (Fig.4F, Appendix S3).

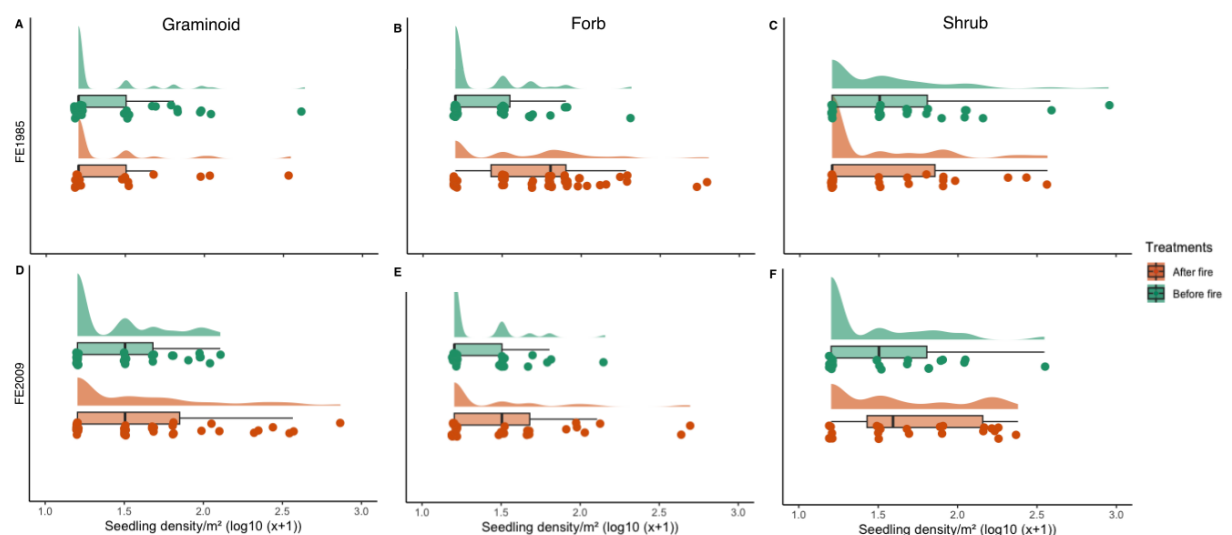


Figure 4. Seedling density (m^2 , mean $\pm$ SE) of different growth forms (graminoid, forb, shrub) which emerged from the seed bank from sites with different fire histories: FE1985 – fire exclusion for more than 30 years (A-C), and FE2009 – fire exclusion for 12 years (D-F), before (BF) and after prescribed fires (AF) in open savannas of the Cerrado in Southeastern Brazil.

Seed bank diversity

For both sites (FE1985 and FE2009) we identified a total of 33 species germinating from the seed bank, belonging to 16 families and 32 genera (see Appendix S1). At FE1985, 25 species were identified, while at FE2009 we found 20 species (see Appendix S1) with a high species co-occurrence (50% species similarity, Sørense Index). At FE1985 and FE2009, the monocot/eudicot ratio was 1:2 and 1:1, respectively. Furthermore, the composition of eudicot species from the seed bank was significantly higher in FE1985 ($p < 0.001$). The seed bank composition significantly differed between treatments (BF and AF) in both sites (see Appendix S4). In general, approximately 20% of the total species only emerged from the seed bank after fire, while only 8% of species exclusively emerged from the seed bank before fire (see also Appendix S1).

The species composition at FE1985 was different from the one found at FE2009 in both treatments (BF and AF, Fig.5; $p = 0.002$ – Appendix S4). The similarity between species composition was low before fire even with some co-occurring species such as: *Phyllanthus orbiculatus*, *Baccharis dracunculifolia*, *Achyrocline satureioides*, *Sporobolus aeneus*, *Axonopus marginatus* and *Axonopus pressus* (Sorensen Index = 0.189 – Appendix S4). On the other hand, the similarity between FE1985 and FE2009 communities was higher after fire reintroduction (Sorensen Index = 0.250 – Appendix S4).

The seed bank at FE1985 showed a higher number of species that only occur at FE1985 (10 species: *Bidens gardneri*, *Croton glandulosus*, *Peltaea polymorpha*, *Desmocelis villosa*, *Melinis repens*, *Cordieira obtusa*, *Borreria sp.*, *Hexasepalum teres*, *Lippia origanoides* and Morfospecies 1- see Appendix S1). Fire stimulated different species to be recruited from the seed bank when compared to ones recruited before fire at FE1985 ($p < 0.001$ – Appendix S4). The shrub species *Desmoscelis villosa* (Melastomataceae) was recruited only before fire, while the forb *Hexasepalum teres* (Rubiaceae) was recruited only after fire (Fig.5). The species composition showed low

similarity between treatments (BF and AF, Sorensen Index = 0.218 – Appendix S4) at FE1985.

The seed bank composition was significantly different between treatments ($p=0.004$ – Appendix S4) at FE2009. Exclusive species from FE2009 were: a graminoid - *Eleocharis capillaceae* (Cyperaceae)- that was recruited only BF, and two forbs - *Ancistrotopis peduncularis* and *Stylosanthes acuminata* (both Fabaceae) - only recruited after fire (See Appendix S1). In addition, *Baccharis dracunculifolia*, *Achyrocline satureioides* (both Asteraceae) and *Axonopus marginatus* (Poaceae) that germinated in both sites were more correlated with FE2009 ($p<0.05$; Fig.5).

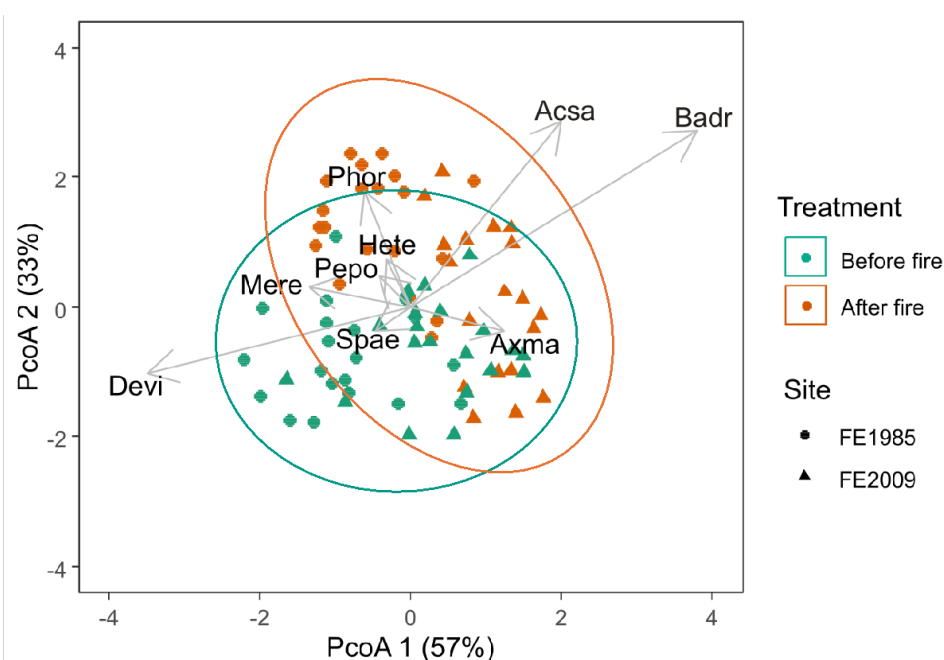


Figure 5. Principal coordinates analysis (PCoA) of seed bank diversity from sites with different fire histories: FE1985 – fire exclusion for more than 30 years, and FE2009 – fire exclusion for 12 years, before and after prescribed fires in open savannas of the Cerrado in Southeastern Brazil. Each point represents the soil sample described by the number of individuals per species. The resemblance measure used among sampling units was Bray-Curtis. Axes values represent the percentage of variance explained. Species abbreviations: Phor – *Phyllanthus orbiculatus*, Hete – *Hexasepalum teres*, Pepo – *Peltaea polymorpha*, Mere – *Melinis repens*, Devi – *Desmoscelis*

villosa, Spae – *Sporobolus aeneus*, Axma – *Axonopus marginatus*, Badr – *Baccharis dracunculifolia*, Acsa – *Achyrocline satureioides*.

4. Discussion

The reintroduction of fire increased seedling recruitment from the seed bank, stimulating post-fire seed germination at both sites, FE1985 and FE2009. Our study showed that Cerrado species germinated more after fire, highlighting the importance of fire for seedling recruitment in tropical savannas. Moreover, the time since last fire appears to have influenced the seed bank composition, with a community more dominated by shrub species the longer the fire exclusion (FE1985), while FE2009 was dominated by graminoids and forbs at FE2009. Fire reintroduction in these sites promoted different effects on the seedling recruitment from the seed bank: fire stimulated graminoid and forb seedlings recruitment in both sites, while there was a significant decrease in shrub seedlings recruitment at FE1985.

Similar to other studies evaluating fire effects on the seed bank (Ghebrehiwot et al. 2012; Cuello et al. 2020), this study highlights that fire stimulates seedling recruitment from the seed bank. In North American deserts, fire influenced the seed bank recruitment in sites with short (~15yr) and long (~30yr) time since last fire (Hosna et al. 2023). Despite some evidence that fire can positively impact seed bank recruitment in some systems, fire can also lead to the decrease of seedling recruitment in others, such as in the semiarid shrublands (Lipoma et al. 2017). Experiments simulating the effects of fire on seed germination trials in the lab such as heat-shock, smoke and combination of them were not successful to predict the real fire effect in seedling recruitment (Cuello et al. 2020). For Cerrado species, few species were recruited after being exposed to fire-related cues, despite being tolerant to the exposure to high temperatures (see Ramos et al. 2016; Daibes et al. 2017; Zironi et al. 2019; Dairel & Fidelis 2020a; Zupo et al. 2021). Although there has been an increase in the number of studies assessing fire effects on

the germination of Cerrado species in the last few years, most studies evaluated the effect of a single driver affecting seed germination (Daibes et al. 2022, but see Zironi et al. 2019). A combination of environmental drivers (Hofmann et al. 2021) may lead to a different response in seedling recruitment since germination traits respond to changes in environmental conditions (Sales et al. 2013).

The longer the time since last fire, the more species were found in the soil seed bank, as it was observed at FE1985. This trend might show the higher importance of the seed bank in the Cerrado when fire is excluded for longer periods. This may indicate a change in the regeneration strategy of Cerrado plants: when fire is frequent, the bud bank plays a major role (Zupo et al. 2021; Pilon et al. 2021; Bombo et al. 2022). However, when fire is excluded for longer periods, there is a decrease in the bud bank (mostly from forbs and shrubs, Bombo et al. 2022). As a consequence, vegetation regeneration and plant persistence will rely more on the seed bank. The higher diversity found in this site is explained by the higher number of eudicot species, mostly shrubs, showing that the process of woody encroachment is occurring, affecting aboveground plant communities (Abreu et al. 2017). At FE1985, long-term fire exclusion leads to a high dominance of woody species in the seed bank and over time, which means that fire-tolerant species may be replaced by fire-sensitive species, such as *Cordia obtusa*, *Desmodium villosa* and *Lippia origonoides* (all shrub species) that were not recruited from the seed bank after fire (see Appendix S1). The only forb that did not recruit from the soil seed bank after fire was *Bidens gardneri*, a perennial species that highly invests in viable seeds as a regeneration strategy (Pinheiro et al. 2022). However, its seeds may not be fire-tolerant and thus, fire would lead to a decrease in its germination.

On the other hand, *Ancistrotopis peduncularis*, *Stylosanthes acuminata*, *Cuphea micrantha*, *Borreria* sp., *Hexasepalum teres* (all forbs) and *Eustachys distichophylla* (graminoid) were recruited from the soil seed bank only after fire. These species are mainly seeders, except for *Borreria* sp. and *Eustachys distichophylla* which are resprouters (Galíndez et al. 2009; Pilon et al. 2021). The recruitment of these species

from the seed bank confirms the important role played by fire and their compatible germination niche originated in a post-fire environment. For example, *Eustachys distichophylla* showed high germination percentage when exposed in photoperiods of 12/12h (light/dark) with different fluctuations of soil temperatures (Pinheiro et al. 2022). In this case, the reintroduction of fire allowed a high temperature fluctuation in the soil surface which may have stimulated the seeds to germinate, being thus an indirect effect of fire (daily temperature fluctuation) that led to an increase in germination of this grass, even only for a few days. Or this species may have fire-related germination cues related to heat shock and smoke, as already shown for other grass species (Ramos et al. 2016; Dairel & Fidelis 2020a). Other species, such as *Ancistrotopis peduncularis*, *Stylosanthes acuminata* are Fabaceae, which probably have physical dormancy and thus, the heat shock (direct effect) or daily temperature fluctuations (indirect effect) might have broken their dormancy, allowing them to germinate more. Some Fabaceae species will germinate even more after being exposed to heat shock, which breaks their dormancy, and smoke, which stimulates them to germinate more (Zirondi et al. 2019).

The reintroduction of fire after long-term fire exclusion highlighted the importance of the seed bank as a source for post-fire regeneration, probably due to a shift in post-fire regeneration strategy from resprouting from the bud bank to germinating from the seed bank. Fire is an important factor assembling vegetation communities in tropical savannas, resulting in the maintenance of the diversity of the species-rich herbaceous layer (Rodrigues & Fidelis 2022). Therefore, fire has an indirect influence on the seed bank dynamics and diversity – seed density and species composition. Despite the seed bank being less recognized and not playing a major role for post-fire recruitment in open savannas of the Cerrado, it is a relevant source for propagules for vegetation regeneration after disturbance events (Bossuyt & Honnay 2008; Kiss et al. 2018; Brown & Cahill 2020), mostly in areas where fire has been excluded for longer periods.

Fire exclusion promotes shifts in aboveground plant community structure and diversity (Parr et al. 2014; Abreu et al. 2017; Archer et al. 2017), but we could already

observe changes in the belowground component of the community (seed bank) before the aboveground community has shifted from an open to a closed state. In fire-prone ecosystems, the main consequence when fire is excluded is the encroachment process (Stevens et al. 2017, Archer et al. 2010). In fire-driven system, fire maintains plant diversity and the plant community structure open, as already described for temperate ones (e.g. tundra and sagebrush communities, Myers-Smith, 2011; Miller et al. 2000; Miller et al. 2005) and tropical ecosystems (e.g. O'Connor et al 2014; Stevens et al. 2017; Abreu et al. 2017; Rosan et al. 2019). In the long-term, changes in aboveground plant community may result in changes in the soil seed bank density and composition (Hopfensperger 2007; Savadogo et al. 2017; Zhao et al. 2021). Time since last fire shows to alter the seed bank species composition in sagebrush communities from the Great Basin (Hosna et al. 2023), showing the importance of fire not only for the aboveground plant communities, but also reflecting its influence on the seed bank. In our study, the longer periods of fire exclusion led to a loss in herbaceous species (graminoid and forb) in the seed bank, whilst the shrub species number increased. The seed bank composition can predict an encroachment process as a consequence of fire exclusion (Abreu et al. 2017; Stevens et al. 2017; Rosan et al. 2019), being supported by the negative effect of fire on shrub species recruitment. Therefore, our results suggest that long intervals between fires enable the replacement of fire-tolerant species seeds by the ones with fire-sensitive seeds. Finally, although FE1985 has not yet shifted to a closed ecosystems, a higher shrub cover in the aboveground vegetation can open gaps in the herbaceous layer leading to a discontinuous fuel load and resulting in lower maximum temperatures and residence time of the fire (Rodrigues et al. 2021). We showed that long-term fire exclusion affected the seed bank species composition and our results suggest that the longer the intervals between fire events, the higher the importance of the seed bank as a source of propagules for post-fire regeneration. Fire mainly increases the herbaceous species recruitment (graminoid and forb), confirming that fire plays a major role on seedling recruitment in the Cerrado, probably through both

direct (e.g. heat shock and smoke) and/or indirect effects (e.g. daily temperature fluctuation and light). Besides that, fire exclusion can lead to changes in the seed bank composition due to the different dominance of growth forms. Fire exclusion increases the woody species with fire-sensitive seeds present in the seed bank, showing a shift from a grassy community (FE2009) to a woody one (FE1985). Hence, understanding the seed bank is crucial for predicting conditions and changes in the aboveground plant communities. Thus, our study brings important results to better understand the processes involved not only on the aboveground, but also the belowground plant community, highlighting how fire exclusion affects how vegetation will regenerate if fire is reintroduced. Finally, this knowledge will help management and restoration practices in open ecosystems of the Cerrado that have been excluded from fire for longer periods.

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Data Accessibility

The data and source codes used are stored within the Mendeley data repository, accessible through the following link: <https://data.mendeley.com/research-data/?search=mariana%20dairiel>.

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Shortened title for Appendices

Appendix S1: List of species sampled in the seed bank

Appendix S2: Seedlings density of eudicot and monocot species in the seed bank

Appendix S3: Seedlings density of different growth forms (graminoids, forbs and shrubs) in the seed bank

Appendix S4: PERMANOVA analysis of seed bank composition

Expanded entries

The reintroduction of fire increased the seedling recruitment from the seed bank, stimulating post-fire seed germination. Cerrado species germinated more after fire, mainly graminoid and forb species, highlighting the importance of fire for seedling recruitment in tropical savannas. Moreover, time since last fire appears to influence the seed bank composition showing a shift in dominance from grassy to woody plant community.



Appendix S1. List of species compounded the seed bank of sites with different fire history (FE1985 and FE2009), before (BF) and after (AF) prescribed fire in open savannas of the Cerrado in southeastern Brazil. Species are classified in growth forms: G – graminoids, F – forbs and S – shrubs. (X) Indicates the presence of the species in the seed bank.

Family	Species	FE1985		FE2009	
		BF	AF	BF	AF
Asteraceae	<i>Baccharis dracunculifolia</i> DC.	X	X	X	X
	<i>Achyrocline satureioides</i> (Lam.) DC.	X	X	X	X
	<i>Chromolaena maximiliani</i> (Schrad. ex DC.) R.M.King & H.Rob.	X	X	X	X
	<i>Campuloclinium macrocephalum</i> (Less.) DC.	X	X	X	X
	<i>Bidens gardneri</i> Baker	X			
Caryophyllaceae	<i>Polycarpaea corymbosa</i> (L.) Lam.	X		X	X
Cyperaceae	<i>Bulbostylis hirtella</i> (Schrad.) Urb.	X	X	X	X
	<i>Eleocharis capillacea</i> Kunth			X	
	<i>Cyperus aggregatus</i> (Willd.) Endl.	X		X	X
Euphorbiaceae	<i>Croton glandulosus</i> L.	X	X		
Fabaceae	<i>Ancistrotropis peduncularis</i> (Kunth) A.Delgado				X
	<i>Stylosanthes acuminata</i> M.B.Ferreira & Sousa Costa				X
	<i>Cuphea micrantha</i> Kunth		X		
Malvaceae	<i>Peltaea polymorpha</i> (A.St.-Hil.) Krapov. & Cristóbal	X	X		
Melastomataceae	<i>Desmoscelis villosa</i> (Aubl.) Naudin	X			
Phyllanthaceae	<i>Phyllanthus orbiculatus</i> Rich.	X	X	X	X
Plantaginaceae	<i>Scoparia dulcis</i> L.	X	X		X
Poaceae	<i>Axonopus marginatus</i> (Trin.) Chase	X	X	X	X
	<i>Axonopus pressus</i> (Nees ex Steud.) Parodi	X	X	X	X
	<i>Eustachys distichophylla</i> (Lag.) Nees		X		X
	<i>Gymnopogon spicatus</i> (Spreng.) Kuntze	X	X		X
	<i>Melinis repens</i> (Willd.) Zizka !	X	X		
	<i>Paspalum hyalinum</i> Nees ex Trin.	X		X	X
	<i>Sporobolus aeneus</i> (Trin.) Kunth	X	X	X	
	<i>Urochloa brizantha</i> (Hochst. ex A. Rich.) R.D.Webster !	X	X	X	X
	<i>Polygala cuspidata</i> DC.	X	X	X	X
Indet.	Morfospecie 1*		X		
Portulacaceae	<i>Portulaca mucronata</i> Link	X	X	X	X
Rapateaceae	<i>Cephalostemon riedelianus</i> Korn.	X	X	X	X
Rubiaceae	<i>Cordia obtusa</i> (K.Schum.) Kuntze	X			
	<i>Borreria</i> sp. G.Mey.*		X		
	<i>Hexasepalum teres</i> (Walter) J.H.Kirkbr.		X		
Verbenaceae	<i>Lippia organoides</i> Kunth	X			

*Were not identified at genus/species level. 1Invasive species.

Supporting information to the paper

Dairel & Fidelis. Fire stimulates seedling recruitment from the seed bank in the Cerrado. *Journal of Vegetation Science*.

Appendix S2. Seedlings density (m^2 ; $\text{mean} \pm \text{SE}$) of eudicot and monocot species in the seed bank of sites with different fire history (FE1985 and FE2009) in both treatments, before (BF) and after prescribed fire (AF) in open savannas of the Cerrado in southeastern Brazil. (*) Indicates statistical differences of eudicot and monocot species among treatments.

Site	Group	Treatments		P value
		BF	AF	
FE1985	Eudicot	77.58 $\pm$ 8.8	72.41 $\pm$ 8.51	0.07
	Monocot	42.38 $\pm$ 11.62	49.78 $\pm$ 18.96	<0.001*
FE2009	Eudicot	40.45 $\pm$ 6.36	70.70 $\pm$ 8.40	<0.001*
	Monocot	41.41 $\pm$ 6.43	93.33 $\pm$ 9.66	<0.001*

Appendix S3. Seedlings density (m^2 ; $\text{mean} \pm \text{SE}$) of different growth forms (graminoid, forb and shrub) in the seed bank of sites with different fire history (FE1985 and FE2009) in both treatments, before (BF) and after prescribed fire (AF) in open savannas of the Cerrado in southeastern Brazil. (*) Indicates statistical differences of each growth form (graminoid, forb and shrub) among treatments.

Site	Group	Treatments		P value
		BF	AF	
FE1985	Graminoid	42.38 $\pm$ 11.62	49.78 $\pm$ 18.96	<0.001*
	Forb	68.17 $\pm$ 29.53	79.35 $\pm$ 12.43	<0.001*
	Shrub	99.2 $\pm$ 45.96	22.4 $\pm$ 3.54	<0.001*
FE2009	Graminoid	41.41 $\pm$ 5.49	93.33 $\pm$ 24.05	<0.001*
	Forb	43.83 $\pm$ 8.12	76.41 $\pm$ 14.05	<0.001*
	Shrub	18.29 $\pm$ 2.29	24 $\pm$ 3.58	<0.001*

Supporting information to the paper

Dairel & Fidelis. Fire stimulates seedling recruitment from the seed bank in the Cerrado. *Journal of Vegetation Science*.

Appendix S4. Pairwise comparisons from PERMANOVA analysis of seed bank composition from sites with different fire histories: FE1985 – fire exclusion for more than 30 years, and FE2009 – fire exclusion for 12 years, before (BF) and after prescribed fires (AF) in open savannas of the Cerrado in Southeastern Brazil. Similarity indices measure by Sorensen Index. Adjusted P values with Bonferroni correction. (*) Indicates statistical differences of seed bank diversity among treatments in each site.

Pairs	Similarity	P value	P- adjusted
BF x AF; FE1985	0.218	0.001*	0.001*
BF x AF; FE2009	0.302	0.004*	0.004*
BF x BF; FE1985 x FE2009	0.189	0.002*	0.002*
AF x AF; FE1985 x FE2009	0.250	0.001*	0.001*

CONSIDERAÇÕES FINAIS

O objetivo desta tese foi avaliar a resiliência de ecossistemas campestres e savânicos do Cerrado frente ao importante fator ecológico modelador dessas comunidades: o fogo. A partir de estudos desenvolvidos em cenários de exclusão de fogo desses sistemas é que podemos investigar o efeito das queimadas mais frequentes sobre a dinâmica e montagem da comunidade vegetal. Tendo em vista que os ecossistemas campestres e savânicos tropicais são modelados não só pela presença de fogo, bem como pelas condições ambientais – principalmente solo e precipitação – escolhemos distintas áreas de Cerrado que apresentam grandes variações nos fatores ambientais e que, principalmente, apresentassem dois históricos de queimas extremos: excluídos do fogo há pelo menos 10 anos e áreas com maior frequência de queimas e que foram recentemente queimadas.

Nesse contexto, no **Capítulo 1**, nós mostramos que a composição de espécies da comunidade vegetal em áreas de Cerrado que estavam excluídas do fogo há longos períodos é diferente daquelas que experimentaram eventos de queima com maior frequência. Além disso, as áreas que queimam com maior frequência apresentam maior riqueza de espécies e uma maior beta-diversidade. Avaliando os componentes da beta-diversidade: turnover (troca de espécies na comunidade vegetal) x nestedness (ganho ou perda de espécies na comunidade vegetal), nós observamos que o componente de nestedness é significativamente maior nas comunidades queimadas, levando a inferir que o fogo leva a um aumento de espécies nessas áreas. Evidenciando assim, o efeito positivo do fogo na comunidade que neste cenário está induzindo o rebrote de espécies que já estavam no sistema subterrâneo dessas comunidades, mas que precisavam do fogo para se estabelecerem na comunidade vegetal aérea. Quanto à estrutura da comunidade, avaliadas através da coleta de biomassa no sistema, observamos que as áreas excluídas têm tendência a um maior acúmulo de espécies arbustivas que podem ser um proxy para processo de adensamento nas comunidades campestres e savânicas do Cerrado. E que ainda, encontramos um maior acúmulo de biomassa morta nessas comunidades, que podem nos

indicar o alto risco de incêndios severos e intensos na comunidade que por sua vez podem influenciar na regeneração da comunidade, bem como na sua resiliência.

Através do **Capítulo 1**, observamos que a exclusão do fogo é um forte fator ecológico que ameaça a resiliência do ecossistema, pois a ausência do fogo leva a uma alteração na estrutura e composição da comunidade vegetal aérea, bem como a perda de biodiversidade. Assim sendo, no **Capítulo 2** buscamos avaliar os efeitos da reintrodução das queimas nesses ecossistemas savânicos tropicais que estavam há longos períodos excluídos do fogo, com diferentes frequências de queima: alta frequência (AF) e baixa frequência (BF). De maneira geral, após 2 anos das queimas, a biomassa aérea e a produtividade do sistema não foram afetados independentemente das áreas. A comunidade vegetal ainda é capaz de recuperar sua biomassa aérea total, contudo a composição da biomassa total por formas de crescimento (graminóides, herbáceas não-graminóides e arbustos) apresentam expressivas diferenças: áreas com maior frequência de fogo apresentam maior contribuição de herbáceas não-graminóides quando comparado às áreas com menor frequência de fogo. Além disso, é importante observar que no segundo ano pós-queima a biomassa de herbáceas não-graminóides cai e há um aumento no acúmulo de biomassa morta, reforçando a necessidade de novas queimas para manter a estrutura e composição da comunidade vegetal aérea.

Quanto à composição da comunidade vegetal, observamos que as áreas com alta frequência (AF) e baixa frequência (BF) já são diferentes entre si antes da queima. Em AF, as áreas queimadas e não queimadas ainda são similares entre si. Por outro lado, a área BF queimada apresenta comunidades vegetais distintas da área BF não queimada. Além disso, a reintrodução do fogo na área BF não foi suficiente para aumentar a diversidade na comunidade vegetal e torná-la mais semelhante às comunidades encontradas nas áreas AF, tanto queimadas como não queimadas. Esse padrão mostra que o tempo de exclusão do fogo na área BF ultrapassou o limite que o sistema ainda consegue se manter resiliente frente ao fogo. Nas áreas BF, provavelmente já poderemos encontrar uma substituição de espécies resistentes por aquelas

sensíveis ao fogo, principalmente devido à perda de órgãos subterrâneos e banco de gemas de espécies que precisam de eventos frequentes de queima.

Uma vez que a resiliência do ecossistema está diretamente associada à disponibilidade e recrutamento de diferentes fontes de propágulo para regeneração da comunidade vegetal após eventos de fogo, no **Capítulo 3** buscamos avaliar o efeito da reintrodução do fogo em áreas com diferentes regimes de fogo (FE1985: 35 anos de exclusão de fogo, 1 queima em 1985; FE2009: 12 anos de exclusão de fogo, 3 eventos de queima sendo a última em 2009) sobre as estratégias de regeneração recrutadas. De maneira geral, o banco de gemas é a fonte de regeneração mais importante para comunidade vegetal mesmo após 35 anos de exclusão do fogo. Apesar da diferença no regime de fogo das áreas estudadas, o banco de gemas apresentou um número de gemas/m² semelhantes entre as áreas, FE1985 e FE2009. Contudo, o número de novos ramos recrutados após a queima foi maior em FE2009. Já o banco de sementes contribui pouco para a regeneração da comunidade vegetal. O banco de sementes tem maior número de sementes/m² em FE1985, apesar disso, na área FE2009 houve maior recrutamento de plântulas após o fogo. No banco de gemas, as espécies graminóides e arbustivas representam grande porcentagem do banco de gemas de ambas as áreas. Após a reintrodução do fogo, as espécies graminóides ainda são as espécies mais dominantes com expressivo recrutamento de novos perfilho via rebrote, evidenciando a resiliência dessas espécies, independente do regime de fogo das áreas. Por outro lado, o banco de sementes é composto principalmente por graminóides e herbáceas não-graminóides em FE2009 e por arbustos e herbáceas não-graminóides em FE1985 e, após a reintrodução do fogo há um expressivo recrutamento de espécies herbáceas em ambas as áreas. O aumento de plântulas de herbácea pode estar conectado com os efeitos diretos e indiretos do fogo no estímulo de germinação da semente que já estava armazenada no banco de sementes do solo. Além disso, houve uma queda expressiva no recrutamento de arbusto via plântulas.

Frente aos resultados obtidos da regeneração da comunidade vegetal através do banco de sementes, desenvolvemos o **Capítulo 4** que buscou avaliar o efeito do fogo no recrutamento de sementes do banco de sementes. Neste capítulo avaliamos as mesmas áreas estudadas no **Capítulo 3**. De forma geral, encontramos que o fogo aumenta o recrutamento de sementes do banco de sementes em ambas as áreas. A reintrodução do fogo promoveu diferentes respostas no recrutamento do banco de sementes: as espécies arbustivas tiveram uma queda na germinação de sementes do banco de sementes nas áreas FE1985, enquanto o recrutamento de espécies arbustivas não foi afetado pelo fogo em FE2009. Esse resultado é exatamente o que vimos através do recrutamento de plântulas no **Capítulo 3**. O tempo desde o último fogo parece influenciar a composição de espécies do banco de sementes, mostrando uma mudança de sistemas mais abertos para sistemas mais fechados. Depois do fogo, mais espécies do banco de sementes foram capazes de germinar, mostrando que os efeitos diretos e indiretos do fogo estão afetando a germinação de sementes que estão armazenadas no banco de sementes.

Por fim, os resultados obtidos neste trabalho ressaltam a importância da presença do fogo em ecossistemas savânicos e campestres do Cerrado. Os resultados aqui discutidos são de alta relevância não só para a ciência, mas também para atores não acadêmicos como gestores de unidades de conservação de Cerrado e tomadores de decisão, subsidiando a implementação do Manejo Integrado de Fogo (MIF), uma vez que a reintrodução do fogo em sistemas abertos controla o acúmulo de material combustível evitando grandes incêndios que podem ser bastante prejudiciais para regeneração da comunidade vegetal. Além disso, promovem um mosaico de comunidades vegetais na paisagem do sistema, visando a manutenção e conservação desses sistemas abertos.