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MIRELA ALCOLEA

O EFEITO DO FOGO NA REMOÇÃO DE SEMENTES NO CERRADO

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Dissertação apresentada para a obtenção do título de Mestra em Ecologia e Recursos Naturais.

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RESUMO GERAL

O fogo é um fator ecológico que modula a dinâmica, diversidade e estrutura de muitos ecossistemas terrestres. Apesar do conhecimento estabelecido sobre o efeito do fogo para algumas comunidades vegetais e animais, importantes interações ecológicas, como a remoção de sementes, são pouco estudadas nesse contexto. Neste estudo, investigamos o efeito do fogo na remoção de sementes em uma área de Cerrado. Através de experimentos em campo em áreas queimadas há um mês, um ano e em áreas controle não queimadas, avaliamos: a remoção de diferentes diásporos (semente de girassol, fruto de *Miconia rubiginosa* e semente artificial); os agentes de remoção; e a relação entre a remoção e características dos microhabitats. O tratamento um mês pós-fogo apresentou maior taxa de remoção para semente de girassol e fruto de *M. rubiginosa*. Já a remoção das sementes artificiais não diferiu entre os tratamentos. As formigas foram o principal grupo removedor. A composição das espécies de formigas que removem sementes variou com a passagem do fogo, porém, a frequência relativa dos diferentes grupos funcionais (baseados na dieta, habitat e tamanho) não foi afetada pelo fogo. As distâncias de remoção realizadas pelas formigas variaram de 4 a 390 cm e não foram afetadas pelo fogo. As características de microhabitat não influenciaram as remoções dos diásporos. De modo geral, nossos resultados mostram que o fogo não altera de maneira expressiva a remoção de sementes no cerrado. Um próximo passo do estudo seria testar o efeito de diferentes regimes de fogo, como diferentes frequências e extensões de área queimada, que poderiam alterar os efeitos na remoção de sementes.

Palavras-chave: Dispersão de sementes. Formigas. Interação planta-animal. Predação de sementes. Savana.

ABSTRACT

Fire may modulates the dynamics, diversity and structure of many terrestrial ecosystems. Although the effects of fire are well studied for some plant and animal communities, the effects of fire on important ecological interactions, such as seed removal, are poorly known. In this study, we investigated the effect of fire on seed removal in a Cerrado. Through field experiments in sites burned one month or one year before, and in unburned control sites, we assessed the removal of different plant diaspores (sunflower seed, fruit of *Miconia rubiginosa* and artificial seed); agents of removal; and the influence of microhabitat features on seed removal. Treatments one-month post-fire had higher removal of sunflower seeds and *M. rubiginosa* fruit. Removal of artificial seeds did not differ among treatments. Ants were the main removers. The composition of ant species that remove diaspores varied with fire, but the relative frequency of different functional groups (based on diet, habitat and body size) were not affected by fire. Removal distance by the ants ranged from 4 to 390 cm, and was not affected by fire. Microhabitat features did not influence removal of diaspores. Overall, our results show that fire does not significantly change seed removal in the cerrado. A desirable next step is to investigate the impact of different fire regimes, such as different fire frequencies and extensions of the burned area, which could alter the effects on seed removal.

Key words: Animal-plant interactions. Ant. Savanna. Seed dispersal. Seed predation.

ÍNDICE

INTRODUÇÃO GERAL

Em diversos ecossistemas terrestres o fogo ocorre de forma natural, sendo uma das principais pressões evolutivas e ecológicas que modulam a composição e distribuição dos organismos (Bond & Keeley, 2005; Pausas & Keeley, 2009). Nesses ecossistemas, o fogo é um dos responsáveis pela estrutura do habitat, favorecendo a presença de ambientes abertos (Bond & Keeley, 2005). As vegetações campestres e savânicas estão intimamente relacionadas com o fogo e ocorrem em diferentes partes da superfície terrestre (Figura 1).

Na região tropical, essas vegetações (*tropical grassy biomes*) variam desde campos abertos a savanas densas, e se caracterizam principalmente pela cobertura de gramíneas C4 e pela ocorrência do fogo (Parr et al., 2014). As interações entre o fogo e as gramíneas são especialmente importantes. O fogo consome a biomassa da cobertura de gramíneas, alterando as taxas de crescimento e sobrevivência das árvores, promovendo a ocupação pelas gramíneas (Parr et al., 2014). O fato de que esses tipos de vegetações dependem de perturbações faz com que elas sejam propensas a mudanças rápidas na cobertura das árvores, tornando-as vulneráveis. Com isso, as mudanças antrópicas nos regimes de fogo são uma das principais ameaças a esses ecossistemas (Probert et al., 2019).

No Brasil, a savana é representada pelo Cerrado (Walter, 2006) (Figura 1). O Cerrado é o segundo maior Domínio Morfoclimático e Fitogeográfico do país, menor apenas que a Floresta Amazônica, ocupando cerca de 2.000.000 km (22% do território brasileiro) (Oliveira-Filho e Ratter, 2002). Está situado principalmente na porção central do Brasil, e se estende por vários estados brasileiros (Figura 2a).

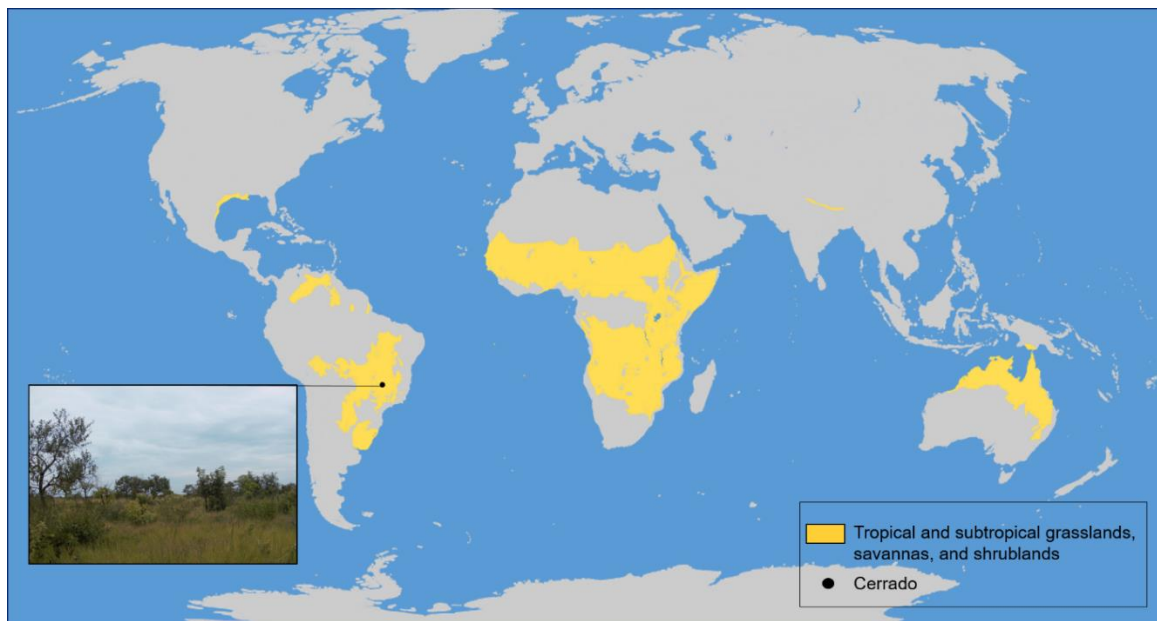Figura 1 - Localização global das vegetações campestres e savânicas tropicais e subtropicais.
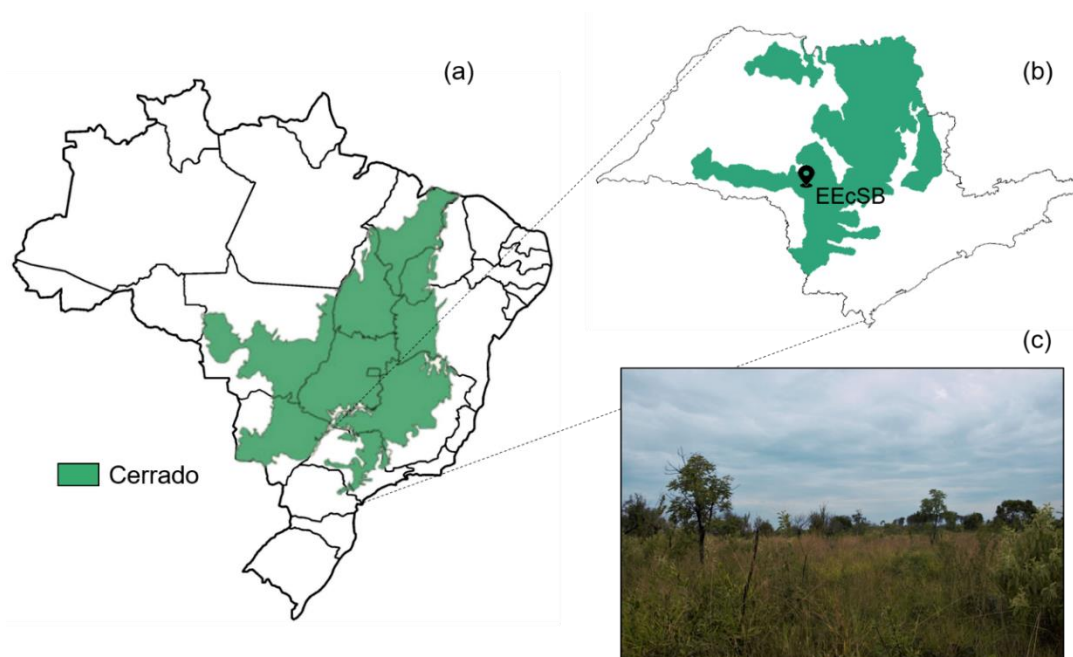Fonte: modificado de https://commons.wikimedia.org/wiki/File:Biome_map_07.svg.

Figura 2 - (a) Principais locais de ocorrência do Cerrado no Brasil; (b) localização do Cerrado
no estado de São Paulo e localização da Estação Ecológica de Santa Bárbara (EEcSB); (c)
exemplo de formação vegetal do Cerrado (campo cerrado) na EEcSB.

O clima no Cerrado caracteriza-se pela presença de verões chuvosos e invernos secos bem definidos, predominantemente com o clima do tipo tropical chuvoso (Aw) segundo o sistema de Köppen (Alvares et al., 2013). A estação chuvosa é concentrada de outubro a março, e a seca entre abril e setembro (Ribeiro e Walter, 2008), com precipitação média anual de 1.500 mm, variando entre 750 mm a 2.000 mm. A temperatura média anual é de 22,5°C, com temperatura média do mês mais frio superior a 18°C (Walter, 2006). As áreas de Cerrado podem ocorrer em altitudes variáveis, abaixo de 300 m até 1.600 m, o que pode gerar grande variação na temperatura (Walter, 2006).

O Cerrado é constituído por um mosaico de fitofisionomias, que englobam formações campestres, savânicas e florestais (Coutinho, 1990; Ribeiro e Walter, 2008). As fitofisionomias podem ser classificadas de diferentes maneiras a partir de diferentes autores (e.g. Coutinho, 1978; Oliveira-Filho e Ratter, 2002; Ribeiro e Walter, 2008). Segundo Ribeiro e Walter (2008), as formações campestres englobam as áreas com predomínio de espécies herbáceas e arbustivas, sem a presença de árvores na paisagem, e incluem as fitofisionomias: campo limpo, campo sujo e campo rupestre. As formações savânicas apresentam áreas com árvores e arbustos espalhados sobre um estrato graminoso, sem a formação de dossel contínuo, e incluem as fitofisionomias: cerrado sentido restrito, parque de cerrado, palmeiral e vereda. Já as formações florestais possuem o dossel contínuo ou descontínuo e predomínio de espécies arbóreas, com as fitofisionomias: mata ciliar, mata de galeria, mata seca e cerradão (Figura 3). A diversidade de ambientes no Cerrado está relacionada a diversos fatores como: sazonalidade climática, topografia, características do solo e incidência de fogo (Furley, 1999; Walter, 2006; Ribeiro & Walter, 2008). Dentre os ambientes citados, formações savânicas e campestres são propensas ao fogo.
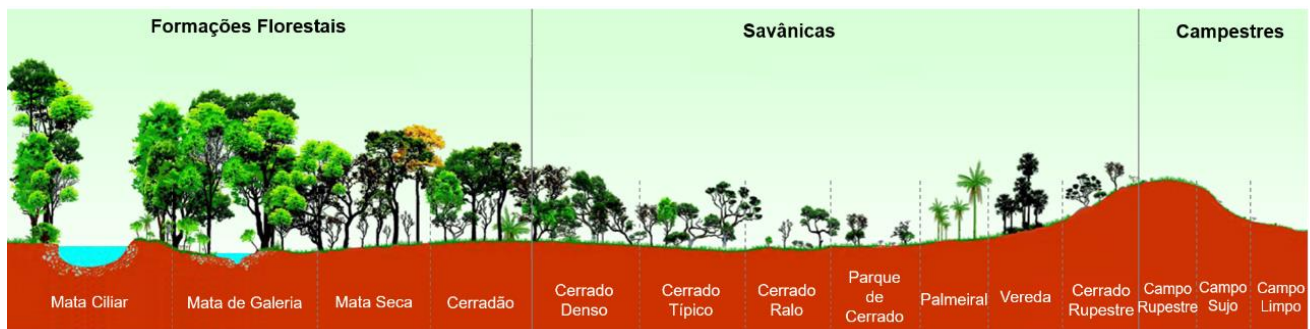
Figura 3 - Esquema das formações vegetais do Cerrado. As linhas contínuas separam as formações florestais, savânicas e campestres, e as linhas tracejadas separam as principais fitofisionomias segundo Ribeiro e Walter (2008). Fonte: modificado de www.embrapa.br/cerrados/colecao-entomologica/bioma-cerrado.

O Cerrado é a savanas que apresenta maior biodiversidade no mundo, com altos níveis de endemismo, sendo um *hotspot* mundial (Myers et al., 2000; Zappi et al., 2015; Murphy et al., 2016). Além de sua riqueza, desempenha diversos importantes serviços ecossistêmicos, como a manutenção de recursos hídricos (Lima & Silva, 2008). Apesar de sua importância, tem pouca proteção ambiental em relação a outros biomas do país (Overbeck et al., 2015), e tem sido extremamente degradado nos últimos anos (Alencar et al., 2020). Entre as maiores ameaças ao Cerrado estão o avanço das áreas de agricultura e de pastagens cultivadas (Alencar et al., 2020). A introdução de espécies gramíneas africanas (principalmente *Urochloa* spp.), relacionadas à pastagem, também gera problemas no Cerrado. Essas espécies de gramíneas são invasoras no Cerrado e podem competir com as gramíneas nativas (Pivello et al., 1999). Outra ameaça está relacionada à modificação dos regimes de fogo naturais no Cerrado, que pode afetar a estrutura dos habitats, as espécies e as dinâmicas ambientais (Durigan & Ratter, 2006; Abreu et al., 2017).

O fogo é um dos principais fatores que modulam os ecossistemas do Cerrado (Coutinho, 1981; Ramos-Neto & Pivello, 2000; Durigan & Ratter, 2006). O fogo no Cerrado ocorre há milhões de anos, estando fortemente ligado à evolução e diversificação das espécies vegetais encontradas no bioma (Simon et al., 2009; Simon & Pennington, 2012). As plantas do Cerrado possuem grande diversidade de adaptações ao fogo, como sementes resistentes a altas temperaturas (Fichino et al., 2016), casca espessa que atua como isolante térmico, e estruturas subterrâneas bem desenvolvidas, como raízes que

permitem a rebrota após a queima (Coutinho, 1990; Simon & Pennington, 2012). O fogo atua também como gatilho na produção de flores e frutos de muitas espécies no Cerrado, podendo influenciar nas dinâmicas populacionais das plantas (Coutinho, 1976; Pilon et al., 2018).

Assim como em espécies vegetais, os animais podem apresentar adaptações ao fogo (Pausas & Parr, 2018). Diversos grupos animais, como pequenos mamíferos, aves, répteis e formigas podem apresentar grande resiliência ao fogo no Cerrado (Durigan et al., 2020). Por outro lado, a supressão do fogo pode levar à modificação do habitat, principalmente com o adensamento da vegetação, favorecendo espécies florestais e resultando na perda de espécies adaptadas a ambientes abertos e processos ecológicos (Durigan & Ratter, 2006; Abreu et al., 2017).

Apesar dos esforços para entender as respostas ao fogo no Cerrado e no mundo, ainda pouco se sabe sobre as interações planta-animal nesse contexto (Arruda et al., 2018; Carbone et al., 2019). A dispersão e predação de sementes por animais são processos ecológicos essenciais na dinâmica populacional das plantas (Wang & Smith, 2002). Diversas espécies de plantas no Cerrado têm a disponibilidade de sementes como fator limitante para o seu recrutamento (Ferreira et al., 2011; Mariano et al., 2019) e a predação de sementes pode diminuir o recrutamento das plantas no Cerrado (Ferreira et al., 2011). Apesar da importância dessas interações, não existem estudos sobre o efeito do fogo na remoção de sementes no Cerrado.

OBJETIVO GERAL

No presente estudo tivemos como objetivo investigar o efeito do fogo na remoção de sementes em uma área de Cerrado.

Objetivos específicos

Caracterizar o efeito do fogo na remoção de sementes considerando:

1) Diferentes tempos após a passagem do fogo (um ano, um mês e controle não queimado);
2) A remoção por vertebrados e formigas;
3) A distância de remoção pelas formigas;
4) As espécies e os grupos funcionais das formigas;

5) A influência do microhabitat na remoção.

ÁREA DE ESTUDO

O estudo foi realizado na Estação Ecológica de Santa Bárbara (EEcSB; 22º48'59" S, 49º14'12" O). A EEcSB possui 2.715 hectares e está localizada no município de Águas de Santa Bárbara, interior do estado de São Paulo (Figura 1b). De acordo com a classificação climática de Köppen, a região está sob o tipo climático Cfa, com clima quente e úmido com inverno seco (Alvares et al., 2013). A temperatura média do mês mais frio é cerca de 18ºC, e a do mês mais quente ultrapassa os 22ºC. A precipitação pluviométrica anual está entre 1000 e 1300 mm. O relevo predominante é do tipo colinoso, paisagisticamente monótono, com baixas declividades (<15%), com altitude entre 600 e 680 m (Melo e Durigan, 2011). O solo predominante é o latossolo, profundos com alto teor de areia, baixo teor de nutrientes e alta saturação de alumínio (Melo e Durigan, 2011).

A EEcSB é ocupada por um mosaico de fitofisionomias do Cerrado, que vão desde formações campestres a florestais. Ao contrário do restante do estado, predominam na unidade as fisionomias abertas, de formações campestres e savânicas, em que as árvores são pequenas e esparsas ou ausentes (Melo e Durigan, 2011; Figura 1c). As diferentes fisionomias são fundamentais para o manejo conservacionista da unidade, e o fogo é em parte responsável pela manutenção das fisionomias abertas.

Ao longo dos anos houve um adensamento da vegetação e aumento da biomassa no local, associados a políticas de supressão do fogo. Esse adensamento resultou na perda de fitofisionomias abertas e espécies vegetais e animais, principalmente nas espécies com preferência por habitats abertos (Abreu et al., 2017). Desde 2015, experimentos com o uso controlado do fogo vêm sendo realizados na EEcSB (Figura 4), em diferentes extensões e fisionomias (Durigan et al., 2020). Os experimentos com o fogo têm o objetivo de estudar os processos de fogo, visando a conservação desses ecossistemas.

Figura 4 - Queima controlada realizada no Cerrado da Estação Ecológica de Santa Bárbara – SP. Foto: Alexander V. Christianini.

ESTRUTURA DA DISSERTAÇÃO

Essa dissertação foi dividida em dois capítulos. No capítulo 1, intitulado "Fire enhances seed removal by animals in a Neotropical savanna", investigamos o efeito do fogo na remoção de diásporos de duas espécies (*Miconia rubiginosa* e *Helianthus annuus*), realizada por vertebrados e formigas. No capítulo 2, intitulado "Seed removal by ants is resilient to disturbance by fire in a Neotropical savanna", avaliamos o efeito do fogo na remoção de sementes realizada pelas formigas, o principal grupo removedor, em diversos aspectos da remoção, como taxa, distância e espécies envolvidas.

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CAPÍTULO 1:

Fire enhances seed removal by animals in a Neotropical savanna

Trabalho redigido nas normas da revista científica *Biotropica*.

1 **Fire enhances seed removal by animals in a Neotropical savanna**

2 _____

3 **Abstract**

4 Seed dispersal and predation by animals often drive plant regeneration. In tropical

5 savannas, such as the Cerrado of Brazil, fire is also a key process in ecosystem

6 dynamics, consuming the lower vegetation strata and killing wildlife, but how fire

7 affects seed-animal interactions is virtually unknown. We investigated the effects of fire

8 on the removal of diaspores of *Miconia rubiginosa* and sunflower *Helianthus annuus* in

9 Cerrado from southeast Brazil. Using plots burned one month or one year before and

10 unburned controls, we assessed the effect of fire on microhabitat structure and diaspore

11 removal by vertebrates and ants. Plots sampled one month after fire had higher overall

12 seed removal than plots one year after fire and unburned controls, with similar

13 responses to both diaspore species. Ants were responsible for most removals. Plots

14 under recent fire (one month) had the most open microhabitats, but microhabitat

15 features did not influence seed removal consistently. Fire increases short-term seed

16 removal, which can influence seed survival, plant regeneration and plant community

17 structure. However, one year is enough to return to the removal rates of unburned

18 control plots, despite microhabitats are still not fully recovered. By increasing frequency

19 and distance of seed removal in the short term, fire events create pulses of opportunities

20 for plant species to increase their populations and enlarge their spatial distribution.

21 However, how different fire intensities and frequencies influence seed fate of different

22 species is still to be investigated.

23 **Keywords:** Ant, Cerrado, granivory, myrmecochory, seed dispersal, seed predation.

24

1. INTRODUCTION

It is well known that fire is a natural major disturbance in many regions of the world (Pausas and Keeley 2009), and one of the most important factors determining distribution, structure and dynamics of vegetation in fire prone ecosystems (Bond & Keeley, 2005). However, anthropogenic modifications in fire regimes, such as those promoted by fire suppression and climate change, can compromise the functioning of these ecosystems (Probert et al., 2019). For instance, long-term fire suppression policies can increase wood biomass and shade in tropical savannas, decreasing habitat suitability for plant and animal savanna specialist taxa (Abreu et al., 2017 and references therein). Aiming at the conservation of fire-prone ecosystems, the use of fire as a management tool has increased in many regions of the world (Driscoll et al., 2010). Fire management is probably required for the long-term conservation of open physiognomies of the neotropical savannas known as Cerrado (Durigan & Ratter, 2016), the second largest vegetation domain in South America (Oliveira-Filho & Ratter, 2006), with high levels of endemism, and one of the hotspots for global conservation (Myers, Mittermeier, Mittermeier, Fonseca & Kent, 2000). Despite its importance, the Cerrado has been degraded fastly (Strassburg et al., 2017). Like any savanna, Cerrado is subject to sporadic and natural occurrence of fires (Coutinho, 1981; Ramos-Neto & Pivello, 2000), and depend on the fire regime to maintain their structure, biodiversity and functioning (Durigan & Ratter, 2006; Abreu et al., 2017). Despite these evidences, Brazil still has no consistent fire policy which foresee the use of fire as a tool for savanna conservation (Durigan & Ratter, 2016). There is a perception of the general public that fire is detrimental to the fauna, and the scarcity of studies call for caution in the recommendation to use fire as a management tool (but see Durigan et al., 2020 and references therein). Moreover, studies addressing the impact of fire on seed predation

50   are relatively scarce (Reed et al., 2004; Parr et al., 2007; Suazo et al., 2013; Setterfield

51   & Andersen 2018) and there is no study about the effects of fire on seed-animal

52   interactions in Cerrado so far.

53   Seed removal by animals, in both mutualistic (dispersal) and antagonistic

54   (predation) interactions, may play a key role in plant population dynamics and spatial

55   distribution. By dispersing diaspores, animals can provide colonization of new sites and

56   increase survival of seeds taken away from the parent plant, and / or decrease predation

57   risk by granivores (Janzen, 1970). Seed predation by granivores can influence plant

58   recruitment and community composition (Janzen, 1970; Hulme, 1998). The likelihood

59   of seed removal is influenced by factors such as the agent of removal, seed traits and

60   habitat/microhabitat in which the seed is found. Ants, rodents and birds are important

61   seed removers in the Cerrado for several plant species (Christianini, Mayhé-Nunes &

62   Oliveira, 2007, 2012; Christianini and Oliveira, 2009; Ferreira, Bruna & Vasconcelos,

63   2011; Rocha-Ortega, Bartimachi, Neves, Bruna, Vasconcelos, 2017). Many plants are

64   adapted for seed dispersal by birds (Kuhlmann & Ribeiro, 2016), several rodents rely on

65   fruit and seed consumption (Vieira & Briani, 2013), and many ants are important seed

66   predators and dispersers for several plants (Ferreira et al., 2011; Christianini et al.,

67   2012; Magalhães, Espírito Santo, Salles, Soares & Oliveira, 2018). While vertebrates

68   may feed primarily on large seeds and in covered microhabitats, ants are likely to carry

69   small seeds and also often forage in open microhabitats (Boulay, Carro, Soriguer &

70   Cerdá, 2009; Suazo, Craig, Vanier & Abella, 2013). Microhabitats with dense

71   vegetation cover can increase protection of granivorous rodents against predation and

72   enhance seed consumption (Manson & Stiles, 1998). On the other hand, open habitats

73   can increase seed discovery, probably increasing ant foraging (Gibb & Parr, 2010).

74   Since fire modify habitat structure (Bond & Keeley, 2005), fire can indirectly

75   influence seed-animal interactions. For instance, a deeper litter reduces the ability of

76   rodents to find seeds, and fire increases seed removal rates in burned environments

77   because fire consumes litter and increases seed detectability (Reed, Kaufman &

78   Kaufman, 2004). Fire in the Australian savanna increases ant seed removal and removal

79   distances, probably because microhabitat simplification increases ant foraging activity

80   (Parr, Andersen, Chastagnol & Duffaud, 2007). Fire can also affect the populations of

81   animals that interact with seeds (Vieira & Briani, 2013; Vasconcelos, Maravalhas &

82   Cornelissen, 2016; Pausas & Parr, 2018) and resource availability (Pilon, Hoffmann,

83   Abreu & Durigan, 2018). As a consequence, seed removal can sometimes decrease in

84   burned areas, and gradually increase with recovery of rodent population and resources

85   (Puig-Gironès, Clavero & Pons, 2018). Nevertheless, information about the effect of

86   fire on seed removal is still limited, and we know little about how persistent are those

87   effects under vegetation recovery after fire.

88   Considering that fire and the time after burning may influence vegetation

89   structure and the animal community, we aim to characterize how fire affects the

90   removal of seeds in the Cerrado considering: 1) different times after fire (one month,

91   one year and unburned adjacent areas); 2) seed removal by vertebrates and ants; 3)

92   influence of microhabitat on seed removal. We hypothesize that 1) seed removal will be

93   higher one month after fire than in the others treatments (following Parr et al., 2007),

94   and one year after fire will have similar seed removal to controls, due to the high

95   resilience and recovery of Cerrado fauna (Durigan et al., 2020); 2) ants will be the main

96   seed removers in all treatments considering the important role of ants in seed removal in

97   the Cerrado (Christianini et al., 2012; Rocha-Ortega et al. 2017); 3) microhabitat traits

98   will influence seed removal, with vertebrates presenting higher removal in covered

99   microhabitats (Manson & Stiles, 1998) and ants presenting higher removal in open

100 microhabitats (Gibb & Parr, 2010).

101

102 **2. METHODS**

103 **1. Study site**

104   The study was carried out in a protected area of Cerrado, the Santa Barbara

105 Ecological Station (hereafter SBES; 22º48'59" S and 49º14'12" W). SBES has 2,715 ha,

106 and is located in the municipality of Águas de Santa Bárbara, São Paulo State,

107 southeastern Brazil. The climate is Köppen Cfa-type, with dry winters and rainy

108 summers, monthly mean temperatures vary between 16° and 24°C and annual mean

109 rainfall is between 1100 and 1300 mm (Melo & Durigan, 2011). The SBES is occupied

110 mostly by different Cerrado physiognomic gradients ranging from open to dense

111 vegetation, but open physiognomies with small and sparse trees occupies the largest areas.

112 Experiments were carried out in campo cerrado, an open physiognomy of Cerrado with

113 trees reaching 2 to 4 meters and covering from 5% to 20% of soil, some shrubs with height

114 below 2 m and a continuous herb layer (Melo & Durigan, 2011; Figure 1a). The most

115 common herbs are *Andropogon leucostachyus* Kunth, *Aristida megapotamica* var.

116 *brevipes* Henrard, *Axonopus pressus* (Nees ex Steud.) Parodi, *Eustachys distichophylla*

117 (Lag.) Nees, *Loudetiopsis chrysothrix* (Nees) Conert and *Sorghastrum minarum* (Nees)

118 Hitchc (Pilon, Assis, Souza & Durigan, 2019). Since 2015 prescribed fire experiments

119 are being carried out at SBES. Prescribed fires were applied annually, in three sites (site

120 1, 2 and 3) located at least 1.7 km from each other, with total burned area varying among

121 sites and years. Burning was set up in the winter, and generally in the morning, whit wind

122   speed below 5 km $^{h-1}$, relative air humidity ranging from 45 to 80% and temperature up

123   to 25 °C (see details in Durigan et al., 2020).

124

125   **2. Sampling**

126   Prescribed burning used for the experiments occurred in July 2018 and 2019

127   (Figure 1b). Experiments were made in two periods after fire, in September 2019

128   approximately one month after fire and between May and June 2019, approximately one

129   year after 2018 burning. All sampling was done in the 2019 dry season. We surveyed ten

130   transects one month after fire (Figure 1c) (five transects in the burned area (hereafter F1)

131   and five transects in the control (Figure 1a) (hereafter C1)). For transects in the burned

132   area, one transect was surveyed in site 1, two transects in site 2 and two transects in site

133   3. We surveyed 16 transects one year after fire (Figure 1d) (eight transects in the burned

134   area (hereafter F2) and eight transects in the control (hereafter C2)). For transects in the

135   burned area, four were surveyed in site 2 and four in site 3. Each transect of burned

136   treatment had a paired unburned control. Locations of transects in burned areas were

137   chosen according to the availability of burned areas. All length of a transect (ca. 50 m)

138   had to be burned, the transect should be at least 10 meters away from the edge of any

139   unburned areas and a distance of at least 70 m from nearest transect. As controls, we

140   considered adjacent areas from burned sites, without fire for at least seven years. Control

141   transects were located with a minimum distance of 20 m from any burned area.

142   To investigate the effect of fire on seed removal we used fruits of *Miconia*

143   *rubiginosa* (Bonpl.) DC. (Melastomataceae) and detached sunflower seeds (*Helianthus*

144   *annuus* L. Asteraceae). *M. rubiginosa* is widely distributed in the Cerrado and produces

145   fleshy fruits (0.12 g), with fruit composed of a high carbohydrate content (87.3% dry

146   mass), small amount of protein (8.3%), lipids (2.8%) and ash (1.6%) (Christianini &

147   Oliveira, 2009). Fruits were collected in March 2019 and frozen until the experiments.

148   Sunflower is an exotic plant in Brazil whose detached seeds (0.06 g) were purchased

149   commercially and are composed of lipids (47.3%), proteins (24%), carbohydrates

150   (19.9%) and ashes (4%) (Watt & Merrill, 1978). We chose two species of diaspores

151   with different chemical compositions (carbohydrate rich and lipid rich) to sample a

152   broad spectrum of ant species, since different groups of ants can be attracted by

153   different chemical compositions of the diaspora (Christianini et al., 2012). Diaspore

154   species were also chosen because of their availability for use in the experiment.

155   We established four sampling stations 10 m apart from each other in each

156   transect. In each sampling station, we placed 10 fruits of *M. rubiginosa* and 10

157   sunflower seeds in the soil, allowing removal by both vertebrates and ants. We also

158   added 10 fruits of *M. rubiginosa* and 10 sunflower seeds under a selective exclosure (a

159   plastic box of height: 9 cm, length: 18 cm, width: 18 cm) with several 1.5 x 1.5 cm

160   openings, allowing only ant access to the seeds. After 24 hr, we counted the number of

161   seeds removed or preyed on at the spot.

162   We measured in all sampling stations microhabitat features of canopy and

163   ground layer vegetation that may influence rodent (Manson & Stiles, 1998) and ant

164   (Gibb & Parr, 2010) foraging. To evaluate the ground layer density, we held a graduated

165   pool vertically above the soil surface in four points (north, south, east and west), one

166   meter from where the seeds were placed. We counted the number of vegetation touches

167   at two levels of the pool: from 0 to 0.5 m and from 0.5 to 1 m from the ground. To

168   estimate canopy and ground layer cover we visually assigned an index for plant cover of

169   0 to 4, as 0 = uncovered, 1 = up to 25% cover, 2 = 25 to 50% cover, 3 = 50 to 75%

170   cover and 4 = over 75 % cover. For canopy cover, estimates were made in a 5 m radius

171 centred at the sampling stations, while for the ground layer a 2 m radius was considered.

172 Additionally, we counted the total number of trees (>10 cm DBH) and shrubs (woody

173 plants branched up to 1 m above ground) within a 2 m radius from the sampling stations

174 (methods adapted from Wikum & Shanholtzer 1978).

175

176 **3. Data analysis**

177   To compare seed removal between treatments, we used generalized linear

178 models (GLM), with Poisson error distribution. We used the number of seeds removed

179 from each sampling station as response variable, and Time (one month and one year),

180 Fire (burned and control), Seed (*M. rubiginosa* and sunflower), and Exclosure (control

181 and exclosure) as fixed factors.

182   To summarize environmental variation between sampling stations we used a

183 principal component analysis (PCA) employing correlation matrices with microhabitat

184 features (Manly, 1997). We verified the relationship between the axis 1 and axis 2

185 extracted from the PCA and microhabitat variables with Pearson's correlations. We

186 used the scores assigned to each sampling station in axis 1 and 2 to test for differences

187 in microhabitats among treatments (F1, C1, F2 and C2) using analysis of variance

188 (ANOVA) followed by Tukey's test for difference between treatments. To assess the

189 influence of microhabitat features on seed removal we regressed the scores of the first

190 two principal components against seed removal through multiple linear regression

191 (Wenny, 2000). To investigate if different agents of diaspore removal prefer some

192 particular traits of microhabitats, regressions were made separately for removal by

193 vertebrates and ants of sunflower seeds and *M. rubiginosa*. To obtain estimates of

194 vertebrate seed removal we calculated the difference between diaspore removal from

195   the paired open control and exclosure treatments. The amount of ant removal was

196   considered as the difference between all removals and vertebrate removal. Analyses

197   were performed with the base stats package in R (R Core Team 2019).

198

199   **3. RESULTS**

200   Overall, 2,971 (71.3%) *M. rubiginosa* fruits and sunflower seeds of 4,170

201   initially placed were removed, with similar removal for Sunflower seeds (52.5% ± 0.2;

202   mean ± SE) and *M. rubiginosa* fruits (47.5% ± 0.2; $P = 0.059$; Table 1). The isolated

203   effects of Fire and Time were not significant, but there was an interaction between Time

204   and Fire (Table 1): transects one month after fire (F1) had higher removal than controls,

205   but this difference disappeared after one year (Figure 2). Exclosures did not influence

206   seed removal (Figure 3), indicating that ants were responsible for most diaspore

207   removal (91.8% of all removals) (Figure 4) while vertebrates had a small contribution to

208   it (8.2%).

209   The first axis extracted from the PCA explained nearly 35% of the variation in

210   microhabitat features. This axis was related to a gradient of increasing vegetation

211   density at the ground layer and was positively correlated with number of touches 0-0.5

212   m and 0.5-1 m above the soil surface and with ground cover, and negatively correlated

213   with canopy cover and number of shrubs (Table 2). The second axis of the PCA was

214   related to a decreasing gradient in canopy cover and explained nearly 25% of the

215   variation in microhabitat traits. Axis 2 was positively correlated with number of shrubs

216   and negatively correlated with canopy cover and number of trees (Table 2). Scores of

217   microhabitat features summarized on axis 1 differed among treatments (F1, C1, F2 and

218   C2; $F = 8.3$, $df = 3$, $P < 0.01$). F1 presented a more open vegetation in relation to F2 and

219   C2, and F2 had a denser vegetation in relation to F1 and C1 (Table 3). Microhabitat

220   features summarized on axis 2 also differed among treatments ($F = 22.4$, $df = 3$, $P <$

221   0.0001). F1 and F2 presented a more open vegetation in relation to C1 and C2. Among

222   all microhabitat variables, F1 had the lowest values, characterizing it as the most open

223   vegetation transects (Figure 5). Despite these differences in microhabitat traits among

224   treatments, there was no consistent influence of microhabitat on seed removal, either by

225   vertebrates (sunflower: $R^2 = 0.01$, $F = 1.73$, $df = 101$, $P = 0.18$; *M. rubiginosa* fruits: R^2

226    $= -0.01$, $F = 0.39$, $df = 101$, $P = 0.68$) or ants (sunflower: $R^2 = 0.00$, $F = 1.10$, $df = 101$,

227    $P = 0.33$; *M. rubiginosa* fruits: $R^2 = -0.01$, $F = 0.52$, $df = 101$, $P = 0.60$).

228

229   **4. DISCUSSION**

230   We experimentally addressed how fire affects the removal of diaspores from a

231   carbohydrate-rich and a lipid-rich plant species over time in Cerrado. Fire increased

232   diaspore removal, but removal rates in unburned vegetation were recovered one year

233   after fire. Fire also increased open microhabitats, but seed removal was quite variable

234   and not related to the microhabitat features measured. Ants were responsible for the vast

235   majority of removals in all treatments.

236   Increased seed removal after fire agrees with results from similar studies in

237   Australian savannas (Parr et al., 2007) and is the opposite related to the sharp declines

238   in diaspore removal after fire in non-flammable ecosystems such as in the Amazon

239   rainforest (Paolucci et al., 2016). Since fire triggers plant sexual reproduction in

240   Cerrado (Coutinho, 1976; Pilon et al., 2018) and seed production can be fast (Fidelis,

241   Rosalem, Zanzarini, Camargos & Martins, 2019), animals may benefit from the

242   exploitation of these ephemeral increases in resources. In the Cerrado, recruitment of

243   several plant species tend to be seed limited (e.g. Ferreira et al., 2011; Mariano, Rebolo

244   & Christianini, 2019), and seed removal, if leading to predation, can limit plant

245   regeneration (Ferreira et al., 2011). Indeed, increases in seed predation after fire

246   enhance recruitment bottlenecks of seed limited plants in Australian savannas

247   (Setterfield & Andersen, 2018). However, seed burial by animals may increase survival

248   to fire, and/or post-fire performance for some seeds and seedlings (Hughes and

249   Westoby, 1992; Boyd, 2001) what may promote a fast reestablishment for plant species

250   after fire. Although not observed in this study, ants (Parr et al., 2007), some rodents and

251   birds may move seeds between unburned and close burned areas, which also could

252   enhance plant colonization/recolonization and vegetation recovery after fire, potentially

253   enlarging plant populations and their spatial distribution.

254   The rate of diaspore removal after fire returns to the levels of control treatments

255   after one year (Figure 2), indicating that this time interval is enough to re-establish the

256   seed removal rates observed in unburned sites. In fire-prone ecosystems, animal and

257   plant species present high resilience, and can have a quick recover after fire (Pausas &

258   Parr, 2018; Durigan et al., 2020). Therefore, our results may be reflecting the short-time

259   changes in plant-animal interactions after fire and the fast return to the pre-fire

260   condition. However, it would be worthwhile to know if the species interacting with

261   seeds are the same across the different treatments, because seed fate may vary with the

262   identity of species in interaction (Christianini et al., 2012; Magalhães et al., 2018).

263   Nevertheless, there is evidence from experiments indicating that richness of several

264   animal groups are not affected one year after fire in Cerrado (Durigan et al., 2020).

265   Thus, it is possible that the identity of species in interaction is maintained after fire.

266   Our results confirm that ants are kept as the most important post-dispersal seed

267   removers in Cerrado (Christianini et al., 2007; 2012; Christianini & Oliveira, 2009;

268 Ferreira et al., 2011; Rocha-Ortega et al., 2017, Magalhães *et al*., 2018) in burned areas.

269 Differences in diaspore removal between ants and vertebrates did not change with fire

270 treatments, indicating that fire does not alter differently the removal by these groups.

271 This is consistent with results from several studies indicating that epigaeic ants with

272 nests underground (as most species removing diaspores in this study, Alcolea &

273 Christianini, unpubl. data) do not show important decreases in abundance with fire

274 (Vasconcelos et al., 2016) and some rodents may find refugees in burrows and nearby

275 vegetation not affected by fire and recolonize burned sites (Puig-Gironès et al., 2018).

276 These results are reinforced by findings that ant, rodent and bird fauna from open

277 savannas are resilient to fire (Durigan et al., 2020). However, it is also important to

278 highlight that soil-nesting ants are often less affected by fire than arboreal ants (Frizzo,

279 Campos & Vasconcelos, 2012; Vasconcelos et al., 2016). Thus, other ant-plant

280 interactions may be affected by fire in different ways, what deserves further

281 investigations.

282   Primary effects of fire on habitat structure includes consumption of plant

283 biomass and increasing habitat openness (Bond & Keeley, 2005). As expected, plots

284 one month after fire (F1) presented the most open microhabitats, with less plant

285 biomass. Cerrado vegetation structure may have a quick re-establishment, as sampling

286 stations one year after fire (F2) presented the most covered plots similar to controls (C2)

287 on PCA axis 1 (Table 3). However, on axis 2, microhabitat features of F2 still differ

288 from controls, and are more similar to F1, indicating that more time would be needed

289 for full recover of microhabitat structure after fire (see Figure 1). Suppression of fire for

290 a long period could increase vegetation density in unburned areas, leading to wood

291 encroachment and loss of savanna specialist taxa (Abreu et al., 2017). Since

292 microhabitat changes may favour certain ant traits (Gibb & Parr, 2010), functional traits

293   (Arnan, Cerdá, Rodrigo & Retana, 2013) and seed removal by the ant community may

294   also be affected by long-term fire suppression. In this way, it would be also worth to

295   understand how seed removal changes with long-term fire suppression, as the balance of

296   seed removal by different animal taxa may also vary with habitats (Camargo,

297   Rodrigues, Piratelli, Oliveira & Christianini, 2019).

298   Fire simplifies microhabitat structure in the short term and can potentially

299   contribute to increase ant activity and foraging efficiency (Parr et al., 2007) and seed

300   detectability by vertebrates (Reed et al., 2004). Indeed, open microhabitats and diaspore

301   removal increase right after fire, but microhabitat features were not related to overall

302   diaspore removal in our study. Therefore, other factors may also influence seed removal

303   such as resource supply (Vasconcelos et al., 2009; Vieira & Briani, 2013). Fire

304   promotes short-term reduction in the abundance of several arthropod taxa that recover in

305   6-24 months (Vasconcelos et al., 2009). These arthropods make the bulk of the diet of

306   many local ant, rodent and bird species (Hölldobler & Wilson, 1990; Ballarini, Frizzas

307   & Marini, 2013; Vieira & Briani, 2013). Shortage of arthropod prey may potentially

308   generate greater demand for food and increase diaspore removal and seed predation in

309   burned areas. The consumption of leaves by fire may also decrease the availability of

310   harvestable material for leaf-cutter ants. This could explain the higher removal of sugar-

311   rich *M. rubiginosa* fruits after fire, since leaf-cutter ants prefer these fruits for fungus

312   growth (Christianini et al., 2012 and references therein). However, we have recorded

313   very few instances of diaspore removal by leaf-cutter ants in this study. Generalist and

314   opportunist ant taxa, such as *Pheidole* and *Ectatomma*, interact with many fallen plant

315   diaspores irrespective of chemical composition (Christianini et al., 2012), and

316   dominated the interactions in our study (Alcolea & Christianini, unpl. Data; Figure 4).

317   Therefore, it is likely that fire-induced changes in resources availability drive the

318 increases in diaspore removal in recently burned sites, irrespective of diaspore chemical

319 composition.

320   This study demonstrates that diaspore removal can be influenced by fire in a

321 short time frame, and one year is enough to re-establish levels of removal rates of

322 unburned sites. However, our results are limited to the time frame, location and species

323 investigated. Since the effects of fire often vary according to habitat, species and fire

324 regimes (Bond & Keeley, 2005; Driscoll et al., 2010; Andersen, 2019), information

325 about how interactions of animals and diaspores vary with different fires in terms of

326 severity, frequency and seasons (Bond & Keeley, 2005) would be worthwhile. Such

327 information may increase our knowledge about the possibilities and constraints to use

328 fire as a management tool aiming at the conservation of open savanna physiognomies.

329

341

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489 **TABLES**

490 Table 1. Response of seed removal by vertebrates and ants to experimental fires in the

491 Cerrado from Águas de Santa Bárbara, southeast Brazil. Factors are Time (one month

492 and one year), Fire (burned and control), Seed (*M. rubiginosa* fruit and sunflower seed),

493 and Exclosure (exclusure of vertebrates and open control). Significant values at P <

494 0.05 are in bold.

Factors	*Z*	*P*
Time	-1.691	0.091
Fire	-0.765	0.444
Seed	-1.891	0.059
Exclosure	-1.700	0.089
Time x fire	2.143	**0.032**
Time x seed	1.090	0.276
Fire x seed	1.700	0.089
Time x exclosure	0.345	0.730
Fire x exclosure	1.304	0.192
Seed x exclosure	-0.874	0.382
Time x fire x seed	-1.362	0.173
Time x fire x exclosure	0.035	0.972
Time x seed x exclosure	0.534	0.593
Fire x seed x e exclosure	-0.198	0.843
Time x fire x seed x exclosure	0.007	0.994

495

496　Table 2. Correlation between microhabitat features measured at sample stations ($n =$

497　104) in experimental transects subjected to fire and controls and PCA axis 1 and 2. The

498　columns show the coefficients of correlation between a given variable and a principal

499　component. Significant values at $P < 0.05$ are in bold.

Variable	Axis 1	Axis 2
Number of touches 0 – 0.5 m	**0.801**	-0.103
Number of touches 0.5 – 1 m	**0.748**	-0.097
Canopy cover	**-0.440**	**-0.726**
Ground layer cover	**0.812**	-0.129
Number of shrubs	**-0.3707**	**0.5276**
Number of trees	-0.1524	**-0.8878**

500

501   Table 3. Scores of sampling stations (mean ± SE) on axis 1 and 2 extracted from a PCA

502   summarizing microhabitat variables measured at different fire treatments (F1 $n = 20$, C1

503    $n = 20$, F2 $n = 32$, C2 $n = 32$). See Table 2 and text for details of microhabitat variables

504   measured. Different letters indicate significant differences after one-way ANOVAs.

| Treatments	Axis 1	Axis 2
F1	-1.1 ± 0.1 a	1.1 ± 0.2 a
C1	-0.4 ± 0.2 a	-1.1 ± 0.3 b
F2	0.7 ± 0.3 b	0.5 ± 0.1 a
C2	0.2 ± 0.2 ab	-0.5 ± 0.2 b

505

506   **FIGURES**
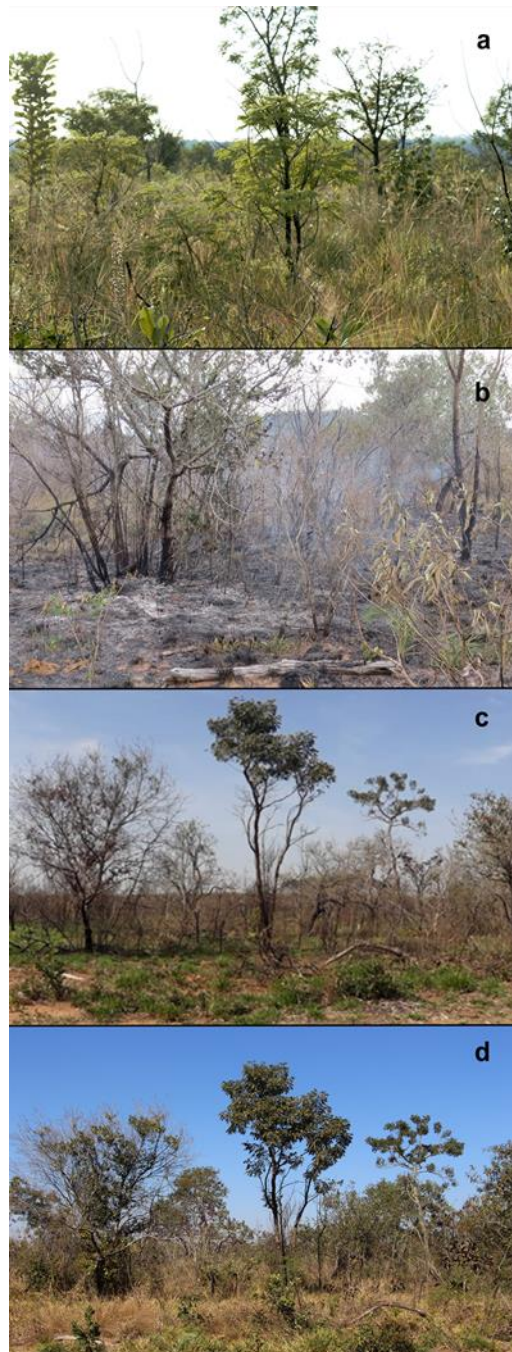
507

508   Figure 1. View of sites where the experiments were performed in the Cerrado from

509   Águas de Santa Bárbara, southeastern Brazil. a) Unburned control; b) immediately after

510   fire; c) one month after fire and d) one year after fire. Photos credits: a, c and d: Mirela

511   Alcolea; b: Alexander V. Christianini.
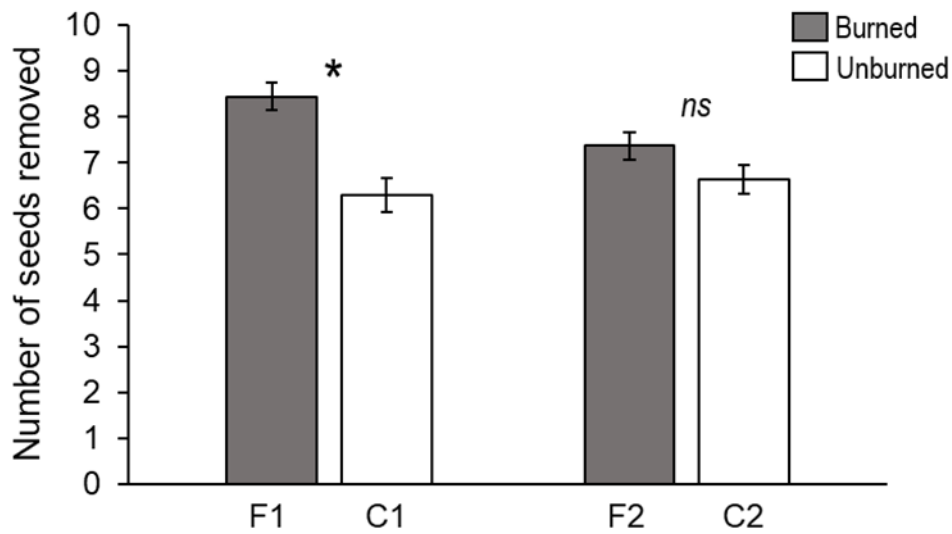
512

513   Figure 2. Mean number of sunflower seeds and *M. rubiginosa* fruit removed by

514   vertebrates and ants in treatments F1 (one month after fire; $n = 80$), C1 (one month after

515   fire control; $n = 80$), F2 (one year after fire; $n = 128$) and C2 (one year after fire control;

516    $n = 128$). Values are means ± standard error. Asterisks represent statistically significant

517   differences among treatments (* $P < 0.05$).

518
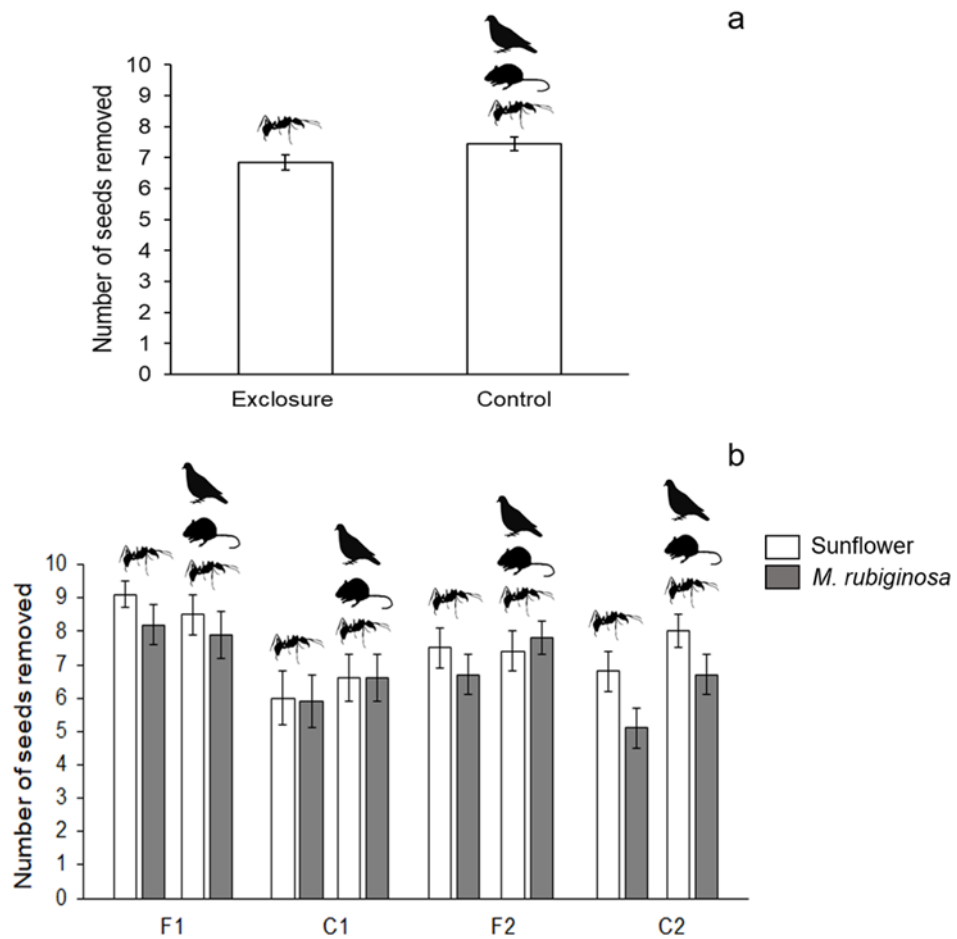
Figure 3. Mean number of sunflower seeds and *M. rubiginosa* fruit removed a) from all exclosure treatment (ant access only; $n = 208$ sampling stations) and control (vertebrate and ant access; $n = 208$) and b) from exclosure and control in treatments F1 (one month after fire; $n = 80$), C1 (one month after fire control; $n = 80$), F2 (one year after fire; $n = 128$) and C2 (one year after fire control; $n = 128$). Ten diaspores of each species were available for animals at each sampling stations. Values are means ± standard error.

525

526 Figure 4. A) *Ectatomma opaciventre* Roger, 1861 (Hymenoptera : Formicidae)

527 removing a sunflower seed and b) *Pheidole oxyops* Forel, 1908 interacting with a fruit

528 of *Miconia rubiginosa* in the Cerrado from Águas de Santa Bárbara, southeastern

529 Brazil. Photos credits: Mirela Alcolea.
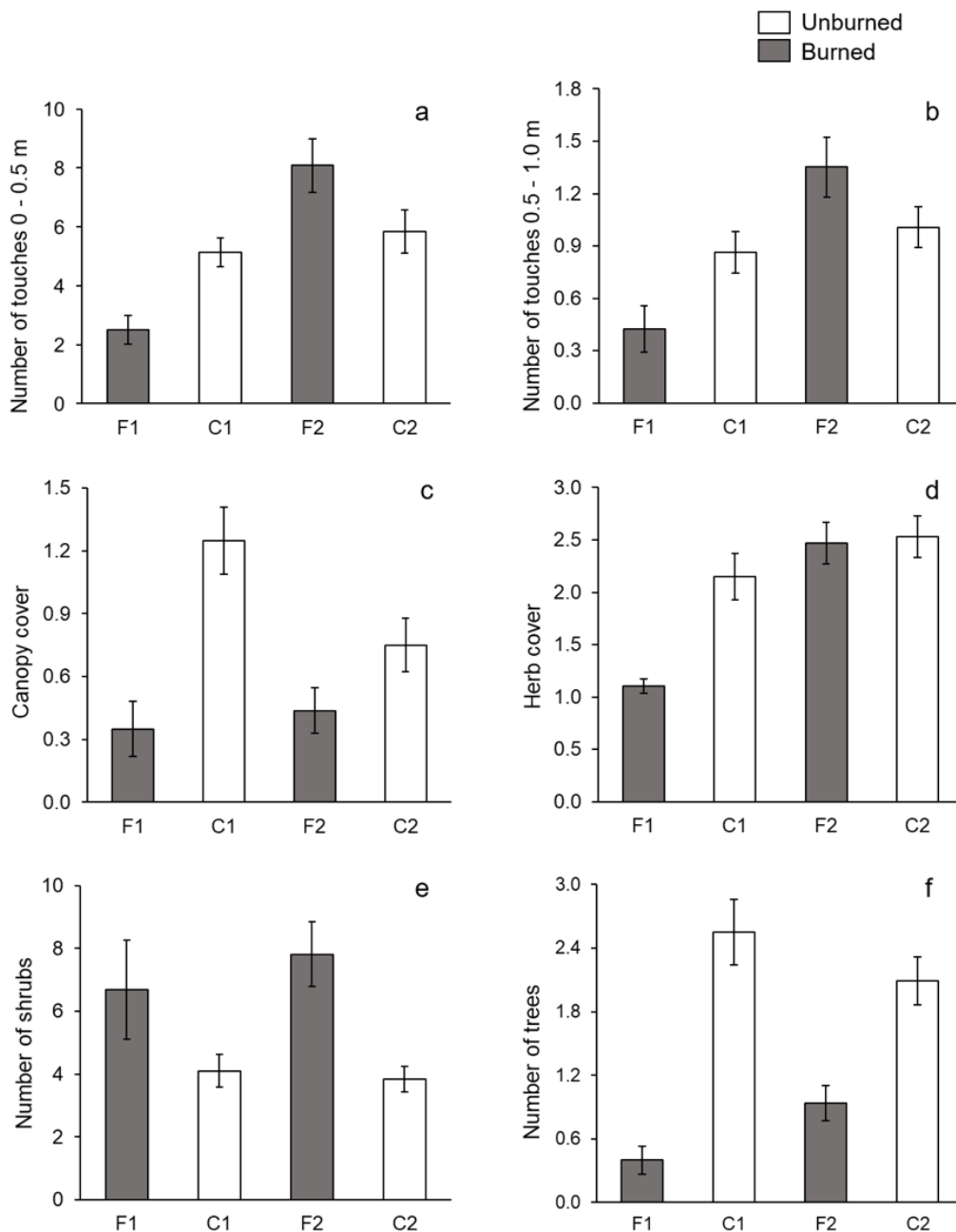
Figure 5. Microhabitat features in treatments F1 (one month after fire; $n = 20$), C1 (one month after fire control; $n = 20$), F2 (one year after fire; $n = 32$) and C2 (one year after fire control; $n = 32$) treatments. For a) number of touches 1 (vegetation touches between 0 to 0.5 m from ground level); b) number of touches 2 (vegetation touches from 0.5 to 1 m); c) canopy cover; d) ground layer cover; e) number of shrubs; f) numbers of trees. Values are means ± standard error. See text for details of how estimates were obtained and Figure 1 for a view of sampling sites.

CAPÍTULO 2:

Seed removal by ants is resilient to disturbance by fire in a Neotropical savanna

Trabalho redigido nas normas da revista científica *Ecological Entomology*.

1 **Seed removal by ants is resilient to disturbance by fire in a Neotropical savanna**

2 _____

3 **Abstract.**

4 1. There is an increasing understanding of the effects of fire on the biota, but the

5   impacts on plant-animal interactions remain much less known. In the Brazilian

6   savanna, the Cerrado, ants are important seed predators and dispersers, but we

7   do not know how disturbance by fire may affect seed removal by them.

8 2. We evaluated the effect of prescribed fires on seed removal by ants in Cerrado.

9   We assessed the rates and distances of seed removal by ants, similarity of ants

10   removing seeds and ant functional groups (based on diet, habitat preference and

11   body size) in sites burned one month or one year before, and in unburned

12   controls. We also measured the relationship between microhabitat features and

13   seed removal.

14 3. Rates and distances of seed removal did not differ among treatments, and seed

15   removal was independent of microhabitat features. Fire incidence reduced the

16   similarity in ant species composition compared to unburned controls, but kept

17   similar ant functional group composition.

18 4. It is likely that the epigaeic Cerrado ant fauna has great resilience to fire, as most

19   epigaeic ant taxa nest in soil allowing colony survival and fast recovery of

20   activity after fire. Fire could have been a strong filter and adaptive pressure on

21   Cerrado ants. Ecological processes driven by ants, such as seed dispersal and

22   predation, may thus be maintained after fire, which may reduce the potential

23   impact of fire on these ant-plant interactions.

24 **Keywords.** Cerrado, dispersal distance, functional groups, granivory, seed dispersal,

25 seed predation.

26 **INTRODUCTION**

27   In fire prone ecosystems, such as savannas, wildfires are among the most

28 important factors determining the composition and distribution of organisms (Bond &

29 Keeley, 2005). One of these ecosystems is the Cerrado, a neotropical savanna that

30 makes the second largest vegetation domain in South America (Oliveira-Filho & Ratter,

31 2006) and a biodiversity hotspot with high levels of endemism (Myers et al., 2000). Fire

32 is one of the most important ecological drivers in the Cerrado (Coutinho, 1990) and fire

33 suppression may lead mesic Cerrado to change into forests (cerradão), causing changes

34 in biodiversity and in ecological processes (Hoffmann & Moreira, 2002; Abreu et al.,

35 2017; Mariano et al., 2019), including loss of savanna specialist species of ants and

36 plants (Abreu et al., 2017). Despite the evidence of the importance of fire, there is no

37 consistent fire policy for the Cerrado, and adding knowledge on fire effects on different

38 taxa and ecological processes are essential for helping to build management policies

39 (Durigan & Ratter, 2016; Schmidt et al., 2018; Durigan et al., 2020).

40   Responses to fire can be quite variable among species, depending on fire

41 regimes and resilience capacity (Bond & Keeley, 2005; Arnan et al., 2013; Pausas &

42 Parr, 2018). Fire can have direct negative effects on animals, such as killing by heat and

43 smoke, or indirect effects, such as decreasing resources availability (Vasconcelos et al.,

44 2009 and references therein). The great fires in Australia between 2019/2020 may have

45 killed an estimate of more than a billion of animals (The Guardian, 2020). Such huge

46 loss of individuals and biomass in a short term may probably have changed several

47 ecosystem processes, such as herbivory and nutrient cycling. However, some plant

48 (Keeley et al., 2011) and animal (Pausas & Parr, 2018) species can have adaptive traits

49 that provide fitness advantages in fire prone environments. For instance, many plants

50 are able to resprout and/or reproduce after burning, enabling then to persist under

51   recurrent fire disturbance events (Pausas & Keeley, 2014). Some animals also benefit

52   from burning, taking advantage of changes in resources or increases in habitat

53   suitability (Pausas & Parr, 2018). It seems several taxa from different plant growth

54   forms, vertebrates (small mammals, birds, lizards, frogs) and ants have great resilience

55   to fire in Cerrado, since they do not decrease in abundance and richness up to one year

56   after fire (Durigan et al., 2020). Despite the effort to understand plant and animal

57   responses to fire, less is known about the impact of fire on insects in general and on

58   their interactions with plants in the Cerrado (Arruda et al., 2018) and in other vegetation

59   domains (Parr et al., 2007; Arnan et al., 2013; Vasconcelos et al., 2016; Carbone et al.,

60   2019).

61   Seed dispersal and predation by animals are key ecological processes in plant

62   population and community dynamics (Wang & Smith, 2002). In the Cerrado, ants

63   usually are the most important agents of seed removal for several plant species

64   (Christianini et al., 2007; Christianini & Oliveira, 2009; Ferreira et al., 2011), and can

65   affect plant recruitment in both negative (Ferreira et al., 2011) or positive (Magalhães et

66   al., 2018) ways. There is evidence that ants play a more positive role in plant

67   regeneration in savannas rather than in mesic forests, probably because ants tend to

68   deposit seeds more often in favourable microsites in the savanna (Camargo et al., 2019).

69   Certain traits of ants may be useful to predict their roles as seed dispersers (Giladi,

70   2006). For instance, predatory ants, like the poneromorphs, often remove seeds away

71   and discard the seed unharmed around the ant nest, what may increase seedling

72   recruitment (Christianini & Oliveira, 2010). Body size is related to dominance status in

73   ant communities and may also influence seed removal distances (Gómez & Espadaler,

74   2013). Habitat preferences may influence the likelihood of ant foraging and

75   removing/depositing seeds from/in different microhabitats. If fire has a biased effect on

76   ants with different combinations of those traits (Arnán et al., 2013), then fire can

77   indirectly influence ant-seed interactions and seed fate.

78   In the Australian savannas, fire increases the abundance and activity of seed-

79   dispersing ants, resulting in increased rates and distances of seed removal (Parr et al.,

80   2007; Beaumont et al., 2012). However, the ant fauna from Australian savannas is

81   mostly derived from ancestral desert taxa, while those from Cerrado has greater affinity

82   with taxa originated from more mesic habitats such as the Atlantic and Amazon forests

83   (Campos et al., 2011; Vasconcelos et al., 2018). Considering that the origin of ant fauna

84   (Campos et al., 2011) and vegetation-fire-climate relationships differ from savannas in

85   Australia, Africa and the Neotropics (Lehmann et al., 2014), it is possible that the

86   responses of ant fauna and their ecosystem services to fire also differ in Cerrado

87   compared to other savannas.

88   Here we evaluated the effect of prescribed fire on the seed removal by ants in

89   Cerrado. We hypothesized that 1) seed removal and removal distances would be greater

90   right after fire (one month) rather than after a year or compared to unburned controls; 2)

91   ant species and functional group composition (based on diet, body size and habitat

92   preferences) will differ among burned and unburned plots, since fire can affect ant

93   species richness and composition in different ways considering savanna or forest taxa

94   (Paolucci et al., 2016; Abreu et al., 2017) ; 3) fire will lead to more open microhabitats,

95   and the microhabitat characteristics will influence seed removal, with open

96   microhabitats presenting higher seed removal by ants (Gibb & Parr, 2010).

97

98   **METHODS**

99   **Study site**

100   This study was conducted on the 2,715 ha protected Santa Barbara Ecological

101   Station, located in southeastern Brazil (SBES; 22º48'59" S and 49º14'12" W). The

102   climate in the region is Köppen Cfa-type, with monthly mean temperatures varying

103   between 16° and 24°C and annual mean rainfall between 1100 and 1300 mm, with dry

104   winters and rainy summers (Melo & Durigan, 2011). The SBES contains mostly

105   Cerrado vegetation, with different physiognomic gradients ranging from open

106   grasslands to forests. Experiments were carried out in campo cerrado, an open

107   vegetation with sparse, 2 to 4 m tall trees covering 5% to 20% of soil, shrubs with

108   height below 2 m and a continuous herb layer (Melo & Durigan, 2011). The most

109   common grasses are *Andropogon leucostachyus* Kunth, *Aristida megapotamica* var.

110   *brevipes* Henrard, *Axonopus pressu*s (Nees ex Steud.) Parodi, *Eustachys distichophylla*

111   (Lag.) Nees, *Loudetiopsis chrysothrix* (Nees) Conert and *Sorghastrum minarum* (Nees)

112   Hitchc (Pilon et al., 2019). The prescribed fire management experiments were applied

113   annually in the winter in SBES. Fire was set up generally in the morning, with wind

114   speed below 5 km h−1, relative air humidity ranging from 45 to 80% and temperature

115   up to 25 °C (see more details in Durigan et al., 2020).

116

Seed removal experiments

118   Prescribed burning used for the experiments reported here occurred in July 2018

119   and 2019, and data was collected approximately one month and one year after burns

120   (Figure 1), in two burned sites (site 1 and 2) located about 2 km from each other. Before

121   the 2018 and 2019 burnings, these sites had already been burned annually or every two

122   years since 2015. As controls, we used paired sites, unburned for at least seven years.

123   To sample ants and diaspore removal responses to fire we established a total of 32

124 experimental plots: 16 experimental plots one month after fire (eight plots in the burned

125 area (hereafter F1) and eight plots in the control (hereafter C1)), as well as 16 plots one

126 year after fire (eight plots in the burned area (hereafter F2) and eight plots in the control

127 (hereafter C2)). Plots were haphazardly set, but burned plots were always at least 10

128 meters away from the edge of unburned savannah, while control plots were set from 20

129 to 100 meters from burned sites. Each plot had a minimum distance of 50 m from each

130 other in the same treatment. All plots were surveyed in the dry season. We sampled four

131 and 12 plots one month after fire in September 2018 and in August 2019, respectively.

132 Plots one year after fire were sampled between May and June 2019.

133   Each plot received a 6 x 6 m grid, where we established 16 sampling stations 2

134 m apart from each other (following Andersen & Morrison, 1998). Each sampling station

135 received a seed on a 2 x 2 cm piece of biodegradable tape. As we needed many seeds,

136 we used artificial seeds in the experiment. Each "seed" was composed of a wood bead

137 (3 mm) surrounded by an artificial lipid-rich aryl (75% hydrogenated vegetable fat,

138 4.8% fructose, 0.5% sucrose, 4.7% glucose, 7% casein, 3% calcium carbonate, 5%

139 maltodextrin), attractive to a wide range of ants (Raimundo et al., 2004). The weight of

140 the seed with the aryl was about 0.01g. The seeds were deposited at the sample stations

141 at the grid and examined for two hours each, in the morning (ca. 7:30 am to 9:30 am)

142 and in the afternoon (4:30 pm to 6:30 pm) to cover the activity of different groups of

143 ants. At the end of the first period, we removed the seeds and replaced them at the

144 beginning of the second period. When we observed a removal event, we recorded the

145 ant species and collected a sample of the specimens. When possible, the ant was

146 followed and the seed deposition site was marked, allowing us to obtain removal

147 distances. Every time the seed was removed during observations, the seed was replaced

148 to increase the cover of interacting ant species. However, only one removal was counted

149   for each sample station for the analysis of the total amount of seed removal. This gave

150   us a maximum of one removal per sample station and 16 removals per plot. For the ant

151   species recorded, ants coming from the same nest were counted once for each sample

152   station, no matter how many seeds those ants removed. Thereby, more than one species

153   could be considered in the same sampling station, if they came from different nests. We

154   only included in our analysis ants that removed the seeds, a behaviour with more

155   implications for seed fate (dispersal, predation) than seed cleaning by ants at the spot.

156   We classified ant species that removed diaspores according to diet and habitat

157   preferences, and body size. Diet preferences followed Leal et al. (2012) classification of

158   functional group categories: 1) Cryptic Predators - small to minute species that nest

159   primarily within the soil, leaf and rotting logs, where they specialise on living

160   arthropods or their eggs; 2) Cryptic Omnivores - small to minute species nesting within

161   the soil, leaf and rotting logs, where they are generalist predators and scavengers; 3)

162   Epigaeic Predators - medium-to large-sized species that forage on the litter surface and

163   are specialist predators of other arthropods; 4) Epigaeic Omnivores - medium-to large-

164   sized species that forage on the litter surface and are generalist predators and

165   scavengers; 5) Arboreal Dominants - highly active and aggressive species with large

166   colony sizes, nesting in trees; 6) Arboreal Subordinates - other tree-nesting species, 7)

167   Opportunists - unspecialized and poorly competitive, ruderal species, 8) Army Ants -

168   highly aggressive, nomadic species with legionary recruitment, 9) Leaf-cutting Attini -

169   highly active and aggressive, polymorphic, species with large colony sizes that use

170   leaves to cultivate a symbiotic fungus; and 10) Non leaf-cutting Attini - monomorphic,

171   non-aggressive species with small colony sizes that use other organic material to

172   cultivate a symbiotic fungus. We aggregate in a single group the Cryptic Omnivores and

173   Epigaeic Omnivores (hereafter Epigaeic Omnivores), and in another one the Leaf-

174   cutting Attini and Non leaf-cutting Attini (hereafter Attini), due to the reduced number

175   of species found on these individual categories (see Results). In habitat preferences,

176   species were classified as habitat generalist, savanna specialist or forest specialist,

177   following Abreu et al. (2017). *Pheidole schwarzmaieri* was classified as a savanna

178   specialist based on Vasconcelos et al. (2018). For body size we grouped ants into three

179   categories: small (< 0.2 cm), medium (between 0.2 and 1.0 cm) or large (> 1 cm)

180   (Christianini et al., 2012).

181   To characterize the effect of fire on microhabitats we estimated canopy cover,

182   ground layer cover, exposed soil and litter cover for each plot by placing a 10 m length

183   measuring tape on the right edge, left edge and in the center of each plot. We walked

184   beside the tape and counted the amount of the tape intercepted by each microhabitat:

185   canopy cover (amount of the tape covered by the projection of a tree canopy), ground

186   layer cover (amount of tape intercepted by herbs), exposed soil and litter (Canfield

187   1941; see Cava et al. 2018 for a similar approach in Cerrado). We then calculated the

188   mean of the three tapes from each variable measured. This allowed us to obtain an

189   average value for each microhabitat feature per plot.

190

191   **3. Data analysis**

192   To assess differences in seed removal between treatments, we used generalized

193   linear models (GLM), with Poisson error distribution, using Time (one month and one

194   year) and Fire (burned and control) as fixed factors. We did not include year of

195   sampling as a separate factor in the analysis, since there was no difference in seed

196   removal between years ($t = 0.9$, $df = 12.58$, $P = 0.20$). Therefore, we opted to join data

197   for both years in a single group to increase power. To assess differences in seed removal

198   distances between treatments we employed two approaches. First, we compared mean

199   distances of removal with a generalized linear model (GLM) with Gaussian error

200   distribution, using Time (one month and one year) and Fire (burned and control) as

201   fixed factors. We also classified distance records in 30 cm categories. We then used

202   Kolmogorov–Smirnov test to compare the frequency distribution of distance categories

203   between treatments.

204   To evaluate the effect of fire on the species richness of seed removing ants we

205   compared the richness of ants removing seeds among fire treatments with one-way

206   analysis of variance (ANOVA), using richness per plot as dependent variable. To

207   evaluate the similarity in composition of ant species removing seeds we calculated the

208   similarity of species recorded in different treatments (F1, C1, F2 and C2) through

209   Sørensen quantitative index, which also consider species abundance (Magurran, 1988).

210   This index is represented by $CN = 2jN / (Na + Nb)$, where CN is the value of Sørensen

211   quantitative index of similarity; Na is the total number of individuals at location A; Nb

212   is the total number of individuals at location B; and 2jN is the lowest sum of the species

213   abundances for each species in the two locations. We evaluated the effect of fire on ant

214   functional groups with Chi-squared tests applied to contingency tables based on

215   presence / absence of functional groups (classified by diet, habitat and size) per plot

216   among treatments (F1, F2 and C).

217   To evaluate if fire changed microhabitat features, first we summarized

218   environmental variation among plots with the aid of a principal component analysis

219   (PCA) employing correlation matrices with microhabitat characteristics (Manly, 1997).

220   We explored the relationships between axis 1 and axis 2 with microhabitat features with

221   Pearson's correlation. We tested for differences in microhabitats among treatments with

222   factorial analysis of variance (ANOVA) using scores from the first and second main

223   components (axis 1 and axis 2). To assess the possible relationship between seed

224   removal and microhabitat features, we regressed the scores of the first two principal

225   components against seed removal through multiple linear regression. Analyses were

226   performed with the base stats package in R (R Core Team 2019).

227

228   **3. RESULTS**

229   Overall, 286 seeds (55.9%) were removed, of 512 initially placed. F1 treatment

230   had 10.7 ± 3.2 (mean $\pm$ SD) seeds removed per plot, followed by C1 (8.9 ± 3.8), C2 (8.7

231   ± 3.4) and F2 (7.4 ± 4.1; Figure 2). Seed removal did not vary with time (one month

232   versus one year after fire; $Z = 0.1$, $df = 31$, $P = 0.93$), fire (burned versus control; $Z = -$

233   1.0, $df = 31$, $P = 0.33$), or with the interaction between time and fire ($Z = 1.5$, $df = 28$, P

234    $= 0.13$). A total of 177 removal distances were recorded in all plots, ranging from 4 to

235   390 cm. The mean overall distance was 87.6 ± 78.0 cm, and about half of the records

236   (51.4%) were up to 60 cm. Removal distances showed no differences between time ($t =$

237   1.0, $P = 0.32$) or fire ($t = 0.1$, $P = 0.96$), and the interaction between fire and time was

238   not significant ($t = 0.2$, $P = 0.81$). The accumulated distribution of distances was

239   variable within treatments and also did not vary between F1 and C1 ($x^2 = 0.03$, $df = 2$, P

240    $= 0.98$) or between F2 and C2 ($x^2 = 0.02$, $df = 2$, $P = 0.98$; Figure 3). However,

241   unburned treatments showed a tendency of more events of seed removal farther than 90

242   cm compared to burned treatments (Figure 3).

243   Ant species identity was recorded in 242 removal events. Twenty-three species

244   from eight different genera performed the removals (Table 1). Among all removals, the

245   genus *Pheidole* dominated the interactions (76.9%), followed by *Ectatomma* (17.3%)

246   and other miscellaneous genera (5.8%) (Figure 4). One single species, *Pheidole oxyops*,

247 was responsible for 32.2% of all seed removals, being presented in all treatments.

248 Species richness showed no difference among fire treatments (F1 = 3.4 ± 1.2 ant

249 species/plot; C1 = 2.7 ± 1.3; F2 = 2.2 ± 1.0; C2 = 3.6 ± 1.2; F = 2.2; df = 3, P = 0.11).

250 However, there was a relatively low similarity in species composition among treatments

251 (F1, C1, F2 and C2), with similarity index between 0.15 and 0.30 (Table 2).

252   Only four functional groups based on diet categories (Leal et al., 2012) were

253 recorded: Attini, Epigaeic Omnivore, Epigaeic Predator and Opportunist. Most species

254 were Epigaeic Omnivores, a result influenced by the high number of records of

255 *Pheidole*. Among habitat preferences, 11 species were habitat generalists and 11

256 savanna specialists. Only 1, *Pheidole vafra*, was a forest specialist recorded only in the

257 controls (C1 and C2; Table 1). For body size, most species (16 species or 69.6%) were

258 of medium size, and well distributed among all treatments (Table 1). Records of

259 different ant groups (based on Diet, Habitat and Body size) did not vary among

260 treatments (Table 3).

261   The first and second axis from the PCA summarized nearly 55% and 30%,

262 respectively, of the variation in microhabitat features. Axis 1 was positively correlated

263 with exposed soil, and negatively correlated with canopy, ground layer, and litter cover

264 (Table 4). Axis 2 was positively correlated with ground layer cover, and negatively

265 correlated with canopy cover and litter (Table 4). Consequently, axis 1 was related to a

266 gradient of microhabitat openness in general, while axis 2 was related to a gradient of

267 soil cover by the ground layer. Microhabitat features summarized on axis 1 differed

268 among treatments (F1, C1, F2 and C2; F = 27.9, df = 3, P < 0.0001) and F1 presented

269 the most opened vegetation (mean score on axis 1 = 2.2 in relation to C1 (-0.8), F2 (-

270 0.4) and C2 (0.9). Microhabitat features summarized on axis 2 did not differ among

271 treatments ($F = 1.90$, $df = 3$, $P = 0.15$). Microhabitats features did not influence seed

272 removal ($R^2 = 0.067$, $F = 2.12$, $df = 2, 29$, $P = 0.138$).

273

274 **4. DISCUSSION**

275   Our results showed that fire has virtually no effects on seed removal by ants in

276 the Cerrado. The amount of seed removal and removal distances do not differ between

277 burned and unburned sites or between a short (one month) and a relatively long (one

278 year) time-frame after fire. Fire seems to have a limited influence on epigaeic ants that

279 remove seeds at ground surface, and no differential effect on ants according to diet,

280 habitat and body size. Recent fire (one month) increases opened microhabitats, but

281 contrary to our hypothesis, there is no clear link between microhabitat features and seed

282 removal by ants.

283   Overall, seed removal rates and distances vary markedly among plots within

284 treatments. This could be due to the spatial variation in the distribution of ant nests. For

285 example, the location of ant nests from *Ectatomma opaciventre* can be associated with

286 certain microhabitats, such as presence of bushes and palms (Pie, 2004). Given the

287 relatively small distances of seed removal (Figure 3), the distribution of ant nests at

288 small scales could cascade to larger variations in seed removal rates and distances

289 among plots. Our mean removal distance (0.88 m) is smaller than the global mean (1.99

290 m) estimate of seed removal by ants (Gómez & Espadaler, 2013). Some studies in the

291 Cerrado show similar removal distances to our study (Christianini & Oliveira, 2010;

292 Magalhães et al., 2018), but larger distances (up to 25 m), and greater means (5 m) are

293 also described (Christianini et al., 2007). The predominance of short distances is

294 probably due to few records of large Ponerinae ants (such as *Pachycondyla*) removing

295 seeds, since removal distance is correlated to ant body size (Gómez & Espadaler, 2013).

296 These ants seem to prefer shaded microhabitats with more tree cover (author's pers.

297 obs.), and were only recorded in unburned controls. Indeed, unburned controls presented

298 more records of distances above 90 cm (Figure 3). The farther removal distances (up to

299 3.9 m in our study) may be especially important for the regeneration of plants that suffer

300 from density-dependent recruitment (Christianini & Oliveira, 2010). The predominance

301 of small removal distances indicates that the scale of influence of ant nests on seeds on

302 the ground will be concentrated in the immediate vicinity of ant nests (Figure 3).

303 Our results contradict previous results about the impact of fire on seed removal

304 in Australian savannas, where fire increases rates and distances of seed removal (Parr et

305 al., 2007; Beaumont et al., 2012). It seems that in Cerrado ants are able to keep similar

306 amounts of interactions with seeds after fire. Epigaeic ants show a high resilience to fire

307 disturbance in Cerrado (e.g. Durigan et al., 2020). However, we detected some changes

308 in the composition of ant species interacting with seeds (Table 2). These changes may

309 have implications for seed fate, as the likelihood of seed dispersal or predation varies

310 with ant identity (Giladi, 2006; Christianini et al., 2012; Magalhães et al., 2018).

311 Despite low similarity of ant species composition among treatments, unburned controls

312 (C1 and C2) have the highest similarities recorded, as well as the plots one year after

313 fire and one year control (F2 and C2). Therefore, it seems fire affect ant composition,

314 but the effect is relaxed with time, indicating a recovery of the ant community along this

315 period (see also Vasconcelos et al., 2009 for examples with several arthropod taxa). The

316 proximity of unburned savanna to burned sites may also facilitate ant recolonization and

317 metapopulation dynamics and allow fast recovery of the fauna (Driscoll et al., 2010). In

318 the future, data about flight dispersal capacity of reproductive stages of ants in Cerrado

319 would allow an evaluation from how far recolonization of disturbed sites may happen.

320   Although ant species interacting with seeds differ to some extent among fire

321   treatments, ant functional traits (considering diet, habitat preference and body size) did

322   not change with fire. This indicates that species replacement is followed by ant taxa

323   with similar traits, and probably redundant effects on seed removal, what may also

324   explain why the amount of seed removal and distances are not affected by fire. These

325   results contrast with the impact of fire in tropical forests, where fire can modify ant

326   functional groups, compromising seed dispersal (Paolucci et al., 2016). Savanna ant

327   communities tend be more resilient to fire than in closed and humid habitats, such as

328   tropical forests (Paolucci et al., 2016; Vasconcelos et al., 2016), probably due to

329   intrinsic habitat and ant species traits (Andersen, 2019). Ants with large body and that

330   occupy high trophic positions, such as large epigaeic predators, or that have their nests

331   more exposed to fire, such as arboreal or litter-dwelling ants, tend to be more sensitive

332   to fire (Frizzo et al., 2012; Paolucci et al., 2016; Vasconcelos et al., 2016). Indeed, ants

333   with these traits were rarely recorded in our study (e.g. *Dinoponera australis* and

334   *Pachycondila striata*). In other parts of the world, such as in Mediterranean ecosystems,

335   fire can alter functional groups of ants, favouring ants with traits such as large colony

336   and worker size, and worker polymorphism (Arnán et al., 2013). Since the origin of ants

337   from Cerrado are biogeographically and evolutionarily associated with rainforests

338   (Campos et al., 2011), fire could have been a strong filter and adaptive pressure on

339   Cerrado ants what may explain the similarity in ant functional traits between fire and

340   control plots in our study.

341   Although fire may have direct effects on ants, killing many individuals, indirect

342   effects, such as changes in habitat structure and microclimate, are often the main drivers

343   on ant community responses to fire (Andersen, 2019). In tropical forests, fire increases

344   habitat openness and lead to decreases in seed removal by ants (Paolucci et al., 2017).

345   Even in tropical forests not disturbed by fire, forest gaps may decrease seed removal by

346   ants (Christianini & Galetti, 2007). Considering that fire is more frequent and remove

347   less plant biomass in savannas, fire has a less dramatic impact on savanna habitat

348   structure compared to forests (Andersen, 2019). Considering that most ants removing

349   seeds are savanna specialist or habitat generalists, it is possible that changes in

350   microhabitats induced by fire were not enough to change abiotic conditions in a way

351   that surpass the physiological tolerance of foraging by these ants (Wiescher et al.,

352   2012). This may explain the absence of microhabitat effects on seed removal in our

353   study.

354   Several animal taxa from Cerrado seems to be resilient to fire (Durigan et al.,

355   2020). Fire has also limited effects on ant interactions with plants bearing extrafloral

356   nectaries in the Cerrado and rupestrian fields (Costa et al., 2018, Fagundes et al., 2018).

357   Our study confirms that an important process that impact plant regeneration in Cerrado,

358   seed removal by ants, is also not heavily affected by fire in terms of removal rates and

359   distances of seed removal. However, fire may change species composition, although

360   keeping the frequency of different ant groups, and more detailed information about seed

361   fate after removal by ants (e.g. predation or dispersal) would be worthwhile to

362   understand the delayed impacts on plant regeneration. A reduction in resource

363   availability (expected immediately following fire) may also turn seed dispersing ants

364   into granivorous ones (Boulay et al., 2005). As the effects of fire may be quite

365   contingent (Bond & Keeley, 2005; Driscoll et al., 2010), a desirable next step would be

366   to test the effects of fire on Cerrado ant fauna subjected to different fire frequencies,

367   intensities and periods of the year, looking at the effects on specific ant taxa and

368   consequences for ants and plant recruitment in the long-term.

369

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532   **TABLES**

533   Table 1. Ant species recorded removing seeds on the floor of Cerrado from Santa

534   Bárbara, southeastern Brazil, and their traits: Diet (A = Attini, EO = Epigaeic

535   Omnivore, EP = Epigaeic Predator and O = Opportunist), Habitat (S = savanna, G =

536   generalist and F = forest), Size (small = < 0.2 cm, medium = between 0.2 and 1.0 cm

537   and large > 1 cm), and the number of seeds removed by them in each treatment: F1 and

538   C1 (one month after fire and unburned control, respectively), F2 and C2 (one year after

539   fire and unburned control, respectively).

Ant species	Diet	Habitat	Size	N seeds removed				
				F1	C1	F2	C2	Total
Acromyrmex aspersus	A	S	medium	2	0	0	0	2
Camponotus rufipes	EO	G	medium	0	0	0	1	1
Dinoponera australis	EP	S	large	0	0	0	1	1
Ectatoma edentatum	O	G	medium	0	1	0	1	2
Ectatomma opaciventre	O	S	large	1	0	0	0	1
Ectatomma permagnum	O	G	large	14	2	7	9	32
Ectatomma planidens	O	S	medium	3	0	1	1	5
Ectatomma tuberculatum	O	S	large	1	0	1	0	2
Mycocepurus goeldii	A	G	medium	0	0	0	2	2
Pachycondila striata	EP	G	large	0	1	0	1	2
Pheidole cyrtostela	EO	S	medium	2	1	0	0	3
Pheidole fracticeps	EO	G	small	1	0	0	1	2
Pheidole gertrudae	EO	G	medium	0	0	14	0	14
Pheidole jelskii	EO	G	medium	0	0	1	0	1
Pheidole oxyops	EO	S	medium	6	21	25	26	78
Pheidole radoszkowskii	EO	G	medium	13	5	1	0	19
Pheidole schwarzmaieri	EO	S	medium	10	3	1	7	21
Pheidole triconstricta	EO	S	medium	15	5	1	12	33
Pheidole vafra	EO	F	medium	0	8	0	1	9
Pheidole sp.03	EO	G	medium	0	3	0	2	5
Pheidole sp.15	EO	S	small	1	0	0	0	1
Trachymyrmex sp.03	A	S	medium	2	0	1	2	5
Trachymyrmex sp.15	A	G	medium	0	1	0	0	1
Total				71	51	53	67	242

540

541   Table 2. Sørensen quantitative index of similarity for ants removing seeds in different

542   treatments (n = 8 for all treatments): F1 (one month after fire), C1 (control of one month

543   after fire), F2 (one year after fire) and C2 (control of one year after fire).

Treatments	F1	C1	F2	C2
F1				
C1	0.18			
F2	0.15	0.25		
C2	0.27	0.30	0.30	

544

545 Table 3. Chi-squared test results for the number of ants recorded in interaction among

546 treatments (F1: one month after fire, F2: one year after fire and C: unburned control)

547 and ant functional groups based on Diet (attini, epigaeic omnivore, epigaeic predator

548 and opportunist), Habitat (savanna specialist, generalist and forest specialist), and Size

549 (small, medium and large).

Ant groups	Df	X^2	P
Diet	6	4.61	0.59
Habitat	4	3.82	0.43
Size	4	2.92	0.57

550

551   Table 4. Microhabitat features (mean ± SD) of plots: measures of canopy, ground layer,

552   soil and litter cover recorded for treatments F1 (one month after fire; n = 8), C1 (one

553   month after fire control; n = 8), F2 (one year after fire; n = 8) and C2 (one year after fire

554   control; n = 8). On the right, the coefficient of correlation between each microhabitat

555   feature at all plots (n = 32) and each axis (Axis 1 and 2) extracted from a Principal

556   Component Analysis (PCA) to summarize microhabitat variation among plots.

557   Significant correlation values ($P < 0.05$) are in bold.

| Variable	F1	C1	F2	C2	Axis 1	Axis 2
Canopy cover	0.25 ± 0.71	2.14 ± 1.48	0.71 ± 0.75	2.11 ± 0.93	**-0.587**	**-0.558**
Ground layer	2.06 ± 1.35	6.06 ± 0.975	6.96 ± 1.34	6.36 ± 0.79	**-0.865**	**0.501**
Exposed soil	7.37 ± 1.79	2.55 ± 1.70	2.21 ± 1.15	2.44 ± 1.40	**0.961**	-0.233
Litter	0.56 ± 0.87	1.39 ± 0.97	0.82 ± 0.47	1.19 ± 0.79	**-0.427**	**-0.774**

558

559 **FIGURES**
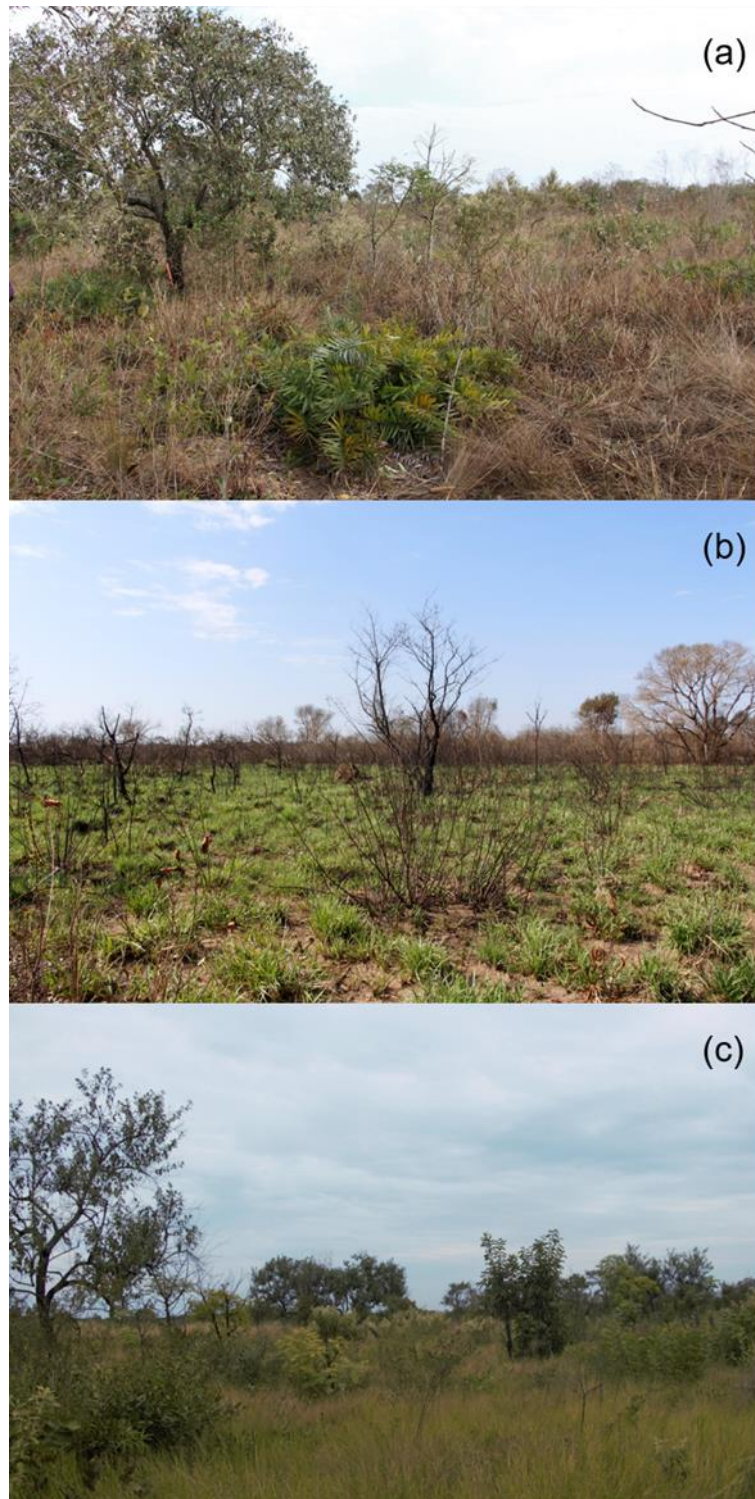
560

561 Figure 1. A sample of sites where the experiments were performed in the Cerrado from

562 Águas de Santa Bárbara, southeastern Brazil. (a) Unburned control; (b) one month after

563 fire and (c) one year after fire. Photo credits: Mirela Alcolea.
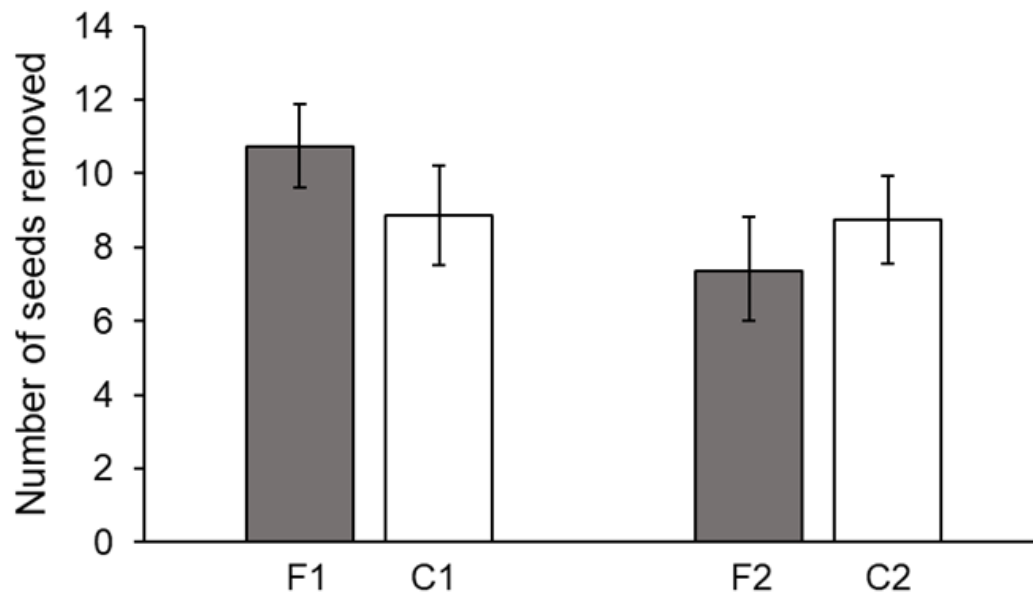
564

565   Figure 2. Number of seeds removed by ants (means ± SE; n = 8 for each treatment)

566   across fire and time. Treatments: F1 and C1 (one month after fire and unburned control,

567   respectively), F2 and C2 (one year after fire and unburned control, respectively).
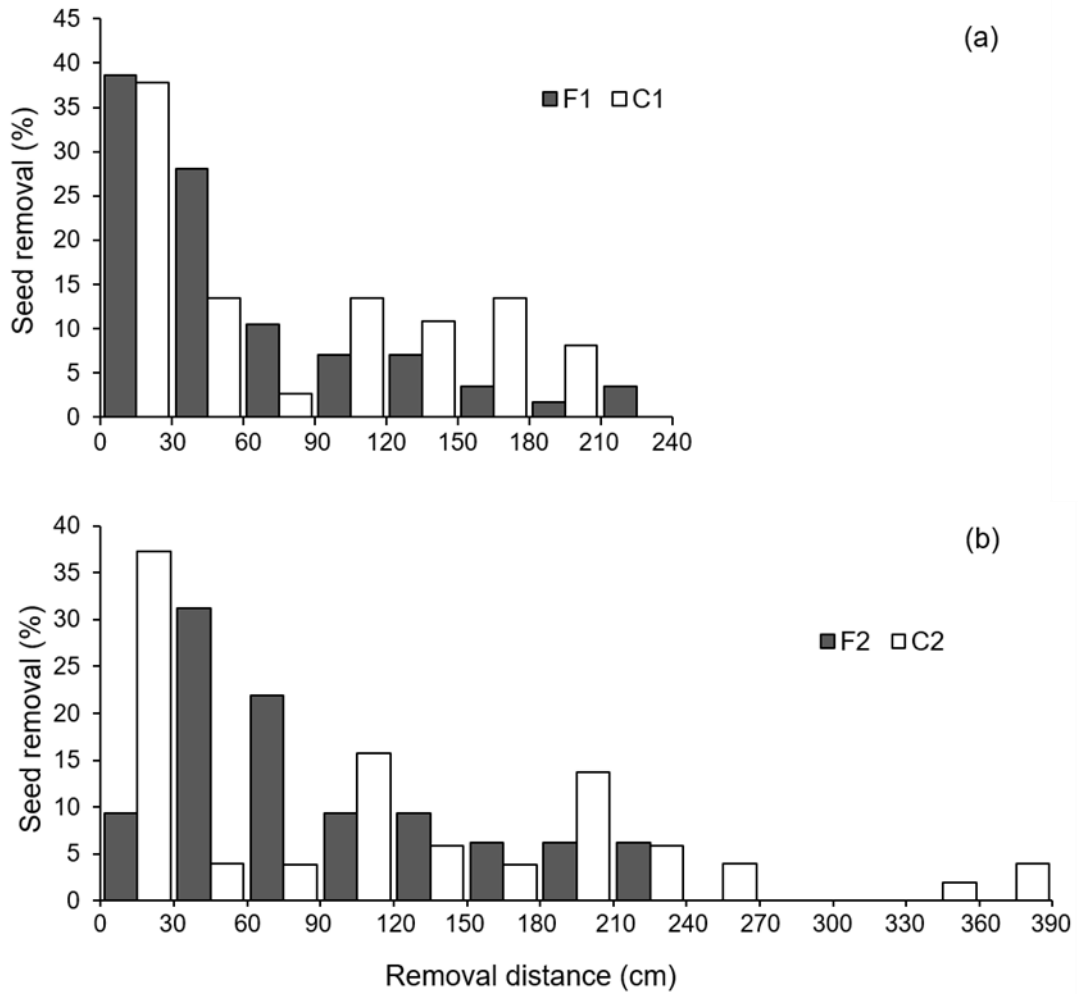
568

569   Figure 3. Frequency distribution of independent distances of seed removal by ants for

570   treatments a) F1 (one month after fire; n = 57), C1 (control of one month after; n = 37)

571   and b) F2 (one year after fire; n = 32) and C2 (control of one year after fire; n = 51).

572

Figure 4. (a) *Pheidole oxyops* (Forel, 1908) and (b) *Ectatomma edentatum* (Roger,

1863) removing an artificial seed in the Cerrado from Águas de Santa Bárbara,

southeastern Brazil. Photo credits: Mirela Alcolea.

CONSIDERAÇÕES FINAIS

Nosso estudo é o primeiro trabalho que investiga o processo ecológico de remoção de sementes no contexto do fogo no Cerrado, e nossos resultados confirmam a resiliência do Cerrado ao fogo nas interações planta-animal. De modo geral, o fogo não apresentou influência expressiva na remoção de sementes. A taxa de remoção das sementes artificiais, a distância de remoção e os grupos funcionais de formigas não variaram com o fogo. Entretanto, a taxa de remoção dos diásporos de *Miconia rubiginosa* e *Helianthus annuus* aumentou em um curto período de tempo pós-fogo. Esse aumento pode influenciar a dinâmica populacional das plantas, afetando o seu recrutamento, porém, o destino das sementes precisa ser melhor investigado. Apesar do fogo causar aumento na remoção de sementes dessas espécies, esse é um efeito de curto prazo, e um ano é suficiente para restabelecer as taxas de remoção. Esses resultados podem ajudar na construção de estratégias de manejo do Cerrado, considerando a necessidade de estabelecer políticas com o uso do fogo para a conservação do Cerrado. Nossos resultados sugerem que o fogo prescrito poderia ser usado sem grande alteração no processo de remoção de sementes. Como próximos passos na construção desse conhecimento, destacamos a investigação da remoção de sementes em diferentes severidades e frequências de fogo, que poderiam alterar os efeitos desse distúrbio.