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Invasão do Cerrado pela Braquiária (*Urochloa decumbens*): impacto na predação de sementes e potencial de disseminação por formigas

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Orientador: Alexander V. Christianini

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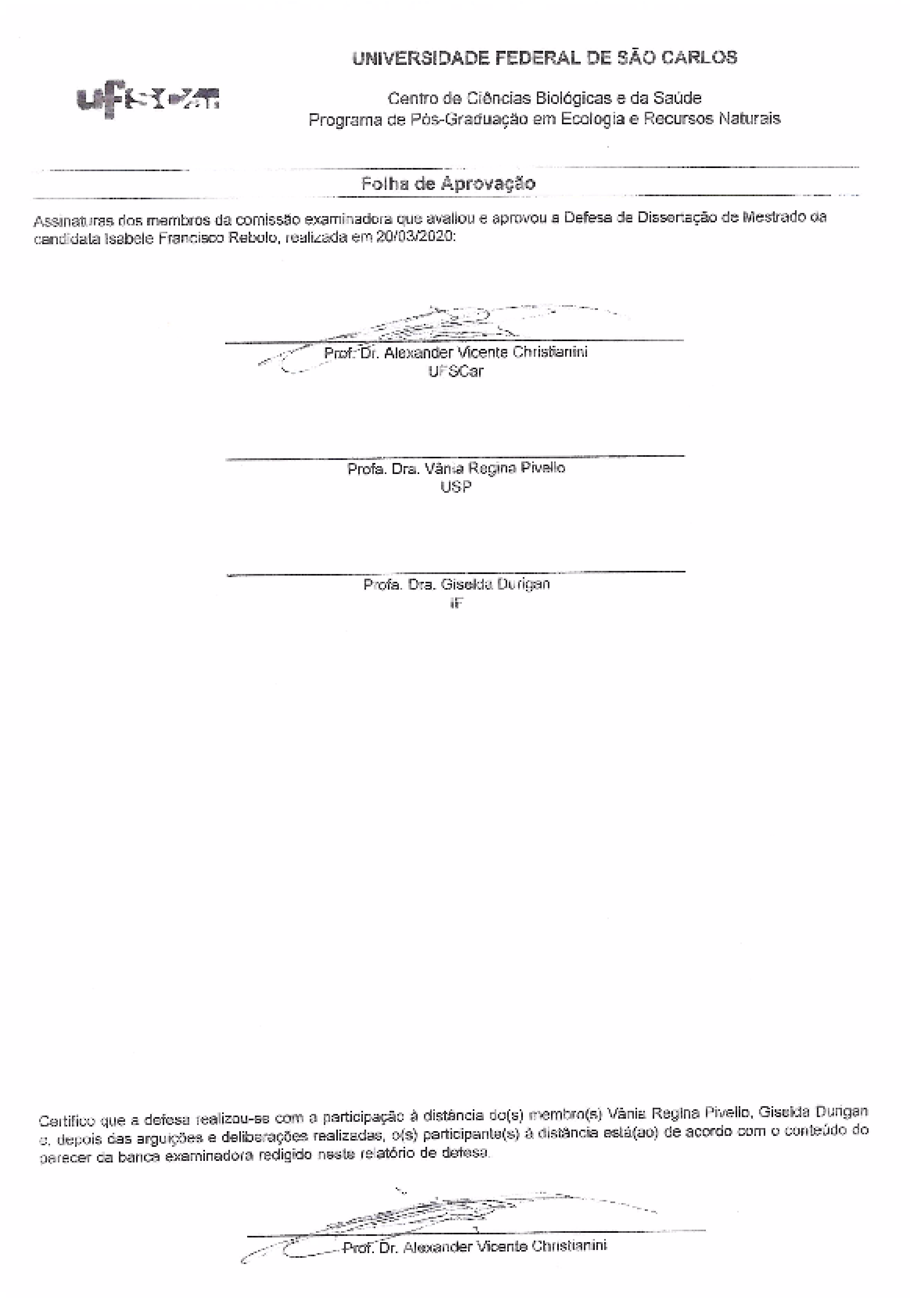

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SUMÁRIO

Resumo geral

Invasões biológicas são uma ameaça para a biodiversidade nativa. Gramíneas africanas (como a braquiária, *Urochloa decumbens*) estão presentes em quase todos os fragmentos de Cerrado, tornando-se um dos principais desafios na conservação do Cerrado. Não há informações sobre o impacto da presença desta invasora na predação de sementes no Cerrado. Roedores, formigas e aves são importantes agentes de predação de sementes, e mudanças na cobertura vegetal podem alterar sua abundância e forrageamento. Sabe-se que *U. decumbens* acumula grande biomassa nas áreas invadidas, proporcionando condições microclimáticas mais amenas, que pode servir como abrigo para agentes de remoção de sementes, o que por sua vez poderia alterar as taxas de predação de sementes. A disseminação de *U. decumbens* ocorre pela ação do vento, propagação vegetativa e pelo trânsito de animais e carros. Não se sabe se formigas atuam na dispersão de *U. decumbens*, e desta maneira, potencializam a invasão. Portanto, os objetivos deste trabalho foram entender se a presença de *U. decumbens* modifica a predação de sementes nativas e da própria *U. decumbens*, e se formigas estão ajudando na disseminação da invasora. Os experimentos foram realizados nas cidades de Itu, Itirapina e Águas de Santa Barbara, durante o verão, em fragmentos de Cerrado. Para testar se a presença de *U. decumbens* altera a predação de sementes, realizamos experimentos de exclusão. Já para verificarmos se formigas estão atuando como dispersoras da gramínea invasora, realizamos testes de tetrazólio nas sementes de *U. decumbens* amostradas nas entradas de formigueiros. Constatamos que locais invadidos apresentam menor predação de sementes, o que sugere que a predação de sementes não seja um dos fatores que afetam a regeneração natural nos locais invadidos por *U. decumbens*. Verificamos que formigas estão atuando como dispersoras da invasora, transportando sementes viáveis para seus ninhos, onde provavelmente há maiores probabilidades de recrutamento em relação ao entorno devido ao acúmulo de nutrientes, e atividade de patrulhamento das formigas em seus ninhos. Portanto, nossos resultados documentam um aumento na complexidade sobre o que sabemos sobre a dinâmica de invasão de *U. decumbens*. e sugere informações importantes para manejo e controle desta gramínea invasora.

Palavras-chave: invasão biológica, dispersão de sementes, predação de sementes

Abstract

Biological invasions are a threat to native biodiversity. African grasses (such as brachiaria, *Urochloa decumbens*) are present in almost all Cerrado fragments, and is one one of the main challenges in the conservation of Cerrado. There is no information about the impacts of this invasive on seed predation in the Cerrado. Rodents, ants and birds are important agents of seed predation, and changes in vegetation cover can alter their abundance and foraging. It is known that *U. decumbens* accumulates hig biomass in the invaded areas, providing milder microclimate conditions, and it may serve as a shelter for seed removal agents, which could change seed predation rates. The spread of *U. decumbens* occurs by wind, vegetative propagation and the transit of animals and cars. It is not known if ants may disperse *U. decumbens*, and in this way, they would enhance the invasion. Therefore, the objectives of this study were: to understand if the presence of *U. decumbens* changes native seed predation and *U. decumbens* seed, and if ants are helping to spread the invasive species. The experiments were done in fragments of Cerrado in Itu, Itirapina and Águas de Santa Barbara municipalities, during the summer. To test whether the presence of *U. decumbens would* alter seed predation, we performed exclusion experiments. To check if ants were acting as dispersers of the invasive grass, we performed tetrazolium tests on the seeds of *U. decumbens* sampled from ant nests. We found that invaded sites have less seed predation, which suggests that seed predation is probably not affecting natural regeneration in places invaded by *U. decumbens*. We verified that ants are acting as dispersers of the invader, transporting viable seeds to their nests, where there is probably higher chance for germination and seedling development in relation to the surroundings due to the accumulation of nutrients, and patrolling activity of ants in their nests.. Therefore, our results suggest an increase in complexity about what we know about the dynamics of *U. decumbens* invasions and suggests important information for the management and control of the invasive grass.

Keyword: biological invasion, seed dispersal, seed predation

Introdução Geral

Espécies invasoras representam uma ameaça para a biodiversidade por conta de seu potencial de modificar o solo, o microclima, alterar o regime de fogo, a estrutura do habitat e substituir espécies nativas (D' Antonio & Vitousek 1992). Atividades antrópicas impulsionaram invasões biológicas, quebrando barreiras biogeográficas e facilitando a dispersão para locais onde não ocorriam previamente (D' Antonio & Vitousek 1992). Invasões biológicas são consideradas como uma das principais causas da extinção de espécies (Gurevitch et al. 2004), por muitas vezes apresentarem vantagem competitiva e dominância em relação às espécies nativas (Valéry et al. 2008). Ainda que não haja a extinção local, espécies invasoras comumente provocam alterações nos grupos funcionais, o que pode modificar o funcionamento do ecossistema (Mason & French 2008). Por exemplo, espécies de plantas invasoras podem influenciar em interações planta-animal, como polinização, dispersão e predação de sementes, funções ecossistêmicas que possuem papéis determinantes para a regeneração e manutenção de população de plantas. (Traveset & Richardson 2006).

A predação de sementes pode interferir na regeneração de plantas por afetar a capacidade local de reposição de indivíduos, de colonização de novos hábitats e distribuição espacial dos regenerantes (Hulme 1998). Predadores pós-dispersão são responsáveis por perdas consideráveis de sementes (Brown & Heske 1990), reduzindo de maneira drástica o recrutamento e diversidade de espécies de plantas (Paine & Beck 2007). Alguns fatores influenciam nas taxas de predação das sementes, como por exemplo: características das sementes, como tamanho, presença de polpa, composição química, e disposição (i.e. se enterrada ou na superfície,) (Hulme 1994; Janzen 1971). Sementes enterradas costumam sofrer menores taxas de predação por roedores devido ao tempo e energia necessários para sua detecção, e recuperação (Hulme & Borelli 2000; Manson & Stiles 1998; Hulme 1994). Além disto, o aumento na cobertura vegetal também pode influenciar nas taxas de predação, por proporcionar refúgio para predadores de sementes (e.g. formigas e roedores), o que pode modificar a abundância e comportamento destes agentes (Orrock et al. 2010), resultando em um aumento de predação de sementes no local. Sabe-se que muitas espécies invasoras são capazes de modificar a cobertura vegetal, podendo influenciar nas taxas de predação de sementes.

Espécies de gramíneas invasoras são importantes ameaças para a biodiversidade nativa, principalmente em ambientes quentes e abertos como campos e savanas (Pivello

1999ª; D'Antonio & Vitousek 1992). Em meados de 1950, gramíneas africanas (e.g. *Urochloa decumbens*) foram introduzidas no Brasil por serem altamente produtivas para pastagem (Williams & Baruch 2000; Hoffmann & Haridasan 2008; Boddey et al. 2004; Pivello et al. 1999a,b; Durigan et al. 2007). *Urochloa decumbens* é uma gramínea africana C4 que apresenta eficiência na competição por recursos, grande produção de sementes, acúmulo de biomassa, resistência a fogo e pisoteio, e facilidade de colonizar ambientes perturbados (D' Antonio & Vitousek. 1992; Pivello et al. 1999 b). Estas características da espécie, junto com as condições climáticas e do solo, facilitaram a disseminação de *U. decumbens* no Cerrado (Pivello 1999a). A presença desta invasora tornou-se uma ameaça à biodiversidade, sendo sua erradicação considerada um dos principais desafios para restauração do Cerrado (Klink & Machado 2005; Durigan et al. 2007). Dentre os impactos advindos da presença de *U. decumbens* podemos citar a constante substituição das gramíneas nativas, devido a sua grande capacidade competitiva. Além disto *U. decumbens* apresenta alelopatia e alta biomassa, que interferem nas taxas de germinação de plantas nativas, geralmente adaptadas à regimes de alta intensidade de luz (Ferreira et al. 2015; Damasceno et al. 2018; Pivelo et al. 1999b). A alta biomassa de *U. decumbens* pode aumentar o sombreamento e umidade do solo, diminuir a temperatura do solo e modificar o regime de fogo local (e.g. Gorgone-Barboza et al. 2015). Agentes de remoção de sementes como aves, formigas e roedores podem ser favorecidos pela maior cobertura vegetal, que proporciona refúgio e condições microclimáticas mais amenas (Orrock et al. 2010). Algumas formigas tendem a evitar áreas com altas temperaturas e baixa umidade (característico de áreas abertas), devido ao aumento no risco de morte por dessecamento (Holldobler & Wilson 1990). Roedores tendem a evitar áreas com menor cobertura de vegetação, pois podem se expor à predadores visualmente orientados (Manson & Stiles 1998; Orrock et al. 2004). Além disso, a composição de aves pode mudar de acordo com a estrutura do local (i.e. cobertura vegetal), por exemplo causando a diminuição de populações de aves granívoras em locais de pastagens com *U. decumbens* (Tubelis & Cavalcanti 2000). Portanto, a maior cobertura vegetal proporcionada pela presença da gramínea invasora pode estar ocasionando modificações no comportamento e abundância dos predadores granívoros, e consequentemente nas dinâmicas de interações planta-animal do local. Em locais invadidos por *U. decumbens*, predadores pós-dispersão (como formigas, aves e roedores) podem estar causando uma perda considerável de sementes (Crawley 1992; Brown & Heske 1990), reduzindo o recrutamento e diversidade de espécies de plantas do Cerrado (Ferreira et al. 2011).

U. decumbens disseminou-se pelo Cerrado através da dispersão por veículos automotivos, propagação vegetativa, vento. *U. decumbens* e provavelmente por animais (e.g. gado). Atualmente é uma invasora em diversos habitats, com cerca de 500.000 km² de áreas de Cerrado ocupados por pastagens dominadas por estas gramíneas (Klink e Machado 2005), e uma fração importante dos fragmentos naturais de Cerrado invadidos (Durigan et al. 2007). O Cerrado ocupa cerca de 21% da área do país, o que corresponde a cerca de 2 milhões de km² (Klink e Machado 2005; Myers et al. 2000). É considerado um dos hotspots mundiais de biodiversidade (Myers et al. 2000; Silva & Bates 2002), e abriga as três maiores bacias hidrográficas da América do Sul (Brasil 2019 / WWF). Mesmo com toda sua importância, o Cerrado sofreu rápido processo de redução de hábitat, com supressões que resultam em mais da metade de seu território transformado em pastos e cultivos (Klink e Machado 2005). Estudos recentes indicam que apenas 20% do Cerrado ainda possui vegetação nativa em estado intacto, e mesmo assim, contém a menor porcentagem de áreas sobre Proteção Integral no país, somando apenas 8,21% de sua área total legalmente protegida com Unidades de Conservação (Brasil 2019/ ICMBio). Ações antrópicas causam perturbações e criam habitats com condições favoráveis para invasões biológicas (D’Antonio & Vitousek 1992), o que facilita a invasão por gramíneas africanas, como tem ocorrido em grande parte das Unidades de Conservação no Cerrado (Pivello et al. 1999a; Durigan et al. 2007).

O processo de invasão consiste em três etapas: dispersão, estabelecimento e disseminação para outras áreas (De Assis 2017). Há poucos estudos com ênfase no processo de dispersão das espécies de gramíneas invasoras. Por ser a primeira etapa do processo de invasão, a dispersão é o melhor estágio para direcionar ações conservacionistas (Puth & Post 2005). Sabe-se que *U. decumbens* apresenta dispersão por chuva de sementes (De Assis 2017; Xavier 2016) e propagação vegetativa dos indivíduos já estabelecidos. A dispersão da gramínea invasora também parece ser favorecida pela presença de bordas, perturbações no ambiente, trânsito de carros e animais (e.g. gado) (Pivello et al. 1999a; Veldman & Putz 2010; Mendonça et al. 2015). Sabe-se que formigas desempenham papéis importantes na dispersão e predação de sementes (Culver & Beattie 1978; Handel 1978; Hanzawa et al. 1988). Muitas vezes a dispersão ocorre de maneira acidental (MacMahon et al. 2000), pois há o descarte de algumas sementes do ninho sem danos, provavelmente por não atenderem às exigências da colônia (Rissing 1986). Quando abandonado o ninho, as sementes podem germinar e se estabelecer (Levey & Byrne 1993). Portanto, formigas, mesmo que granívoras, podem também desempenhar um papel importante na dispersão de *U. decumbens*, mas esta possibilidade é desconhecida até o momento. Compreender os possíveis impactos da presença (cada vez mais constante) da gramínea invasora *U. decumbens* nas interações planta-animal, e se formigas estão potencializando a dispersão das sementes da invasora, podem auxiliar no desenvolvimento de iniciativas de controle mais eficientes de *U. decumbens*. Portanto, nosso objetivo foi avaliar como se dão as dinâmicas de interação planta-animal em um cenário de invasão por *U. decumbens*. O capítulo um teve como objetivo analisar se a presença da *U. decumbens* pode afetar a predação de sementes nativas do cerrado, se sementes enterradas apresentam maiores chances de sobrevivência do que sementes na superfície, quais são os agentes de predação mais importantes, e se sementes de *U. decumbens* sofrem menor predação em locais invadidos pela própria gramínea africana. E o capítulo dois teve como objetivo investigar se formigas estão atuando como dispersoras de *U. decumbens*, identificar as espécies de formigas que carregam sementes de *U. decumbens* para os ninhos, e investigar a escala que as formigas podem estar expandindo a ocorrência da invasora.

Locais de estudo

Os experimentos foram conduzidos em quatro áreas do interior de São Paulo, Sudeste do Brasil, localizados nos municípios de: Itu (23º17’57.67”S e 47º19’37.11”), duas áreas em Itirapina (22º 15’a 22º 15’ S, 47º 45’e 47º 51’ W) e Águas de Santa Bárbara (22º48’59”S e 49º14’12”W) (Figura 1 e 2). As temperaturas variam em média entre 18º e 22º, e pluviosidade em média de 30 mm nos meses mais secos em média 1450 mm nos meses mais chuvosos (SMA 2006; SMA 2011). Todas as áreas de estudo apresentam vegetação de Cerrado, onde encontram-se por exemplo as espécies vegetais *Attalea geraensis*, *Ananas ananassoides*, *Caryocar brasiliensis*, *Aristida jubata* e *Andropogon bicornis* (observação pessoal). Os locais apresentam a presença de gramíneas nativas e gramíneas invasoras (como *U. decumbens*), e vegetações abertas (evitando fitofisionomias de cerradão).

Os experimentos para investigar se a presença de *U. decumbens* está interferindo na predação de sementes foram conduzidos em fragmentos do Condomínio Xapada Itu (na cidade de Itu), na Estação Ecológica de Itirapina e na Estação Ecológica de Santa Bárbara. Já os experimentos para investigar se formigas estão atuando como dispersoras de sementes de *U. decumbens* foram realizados em aceiros na Estação Experimental de Itirapina, sendo que ao percorrer os aceiros é possível notar a presença de ninhos de formigas com sementes de *U. decumbens* nas entradas.

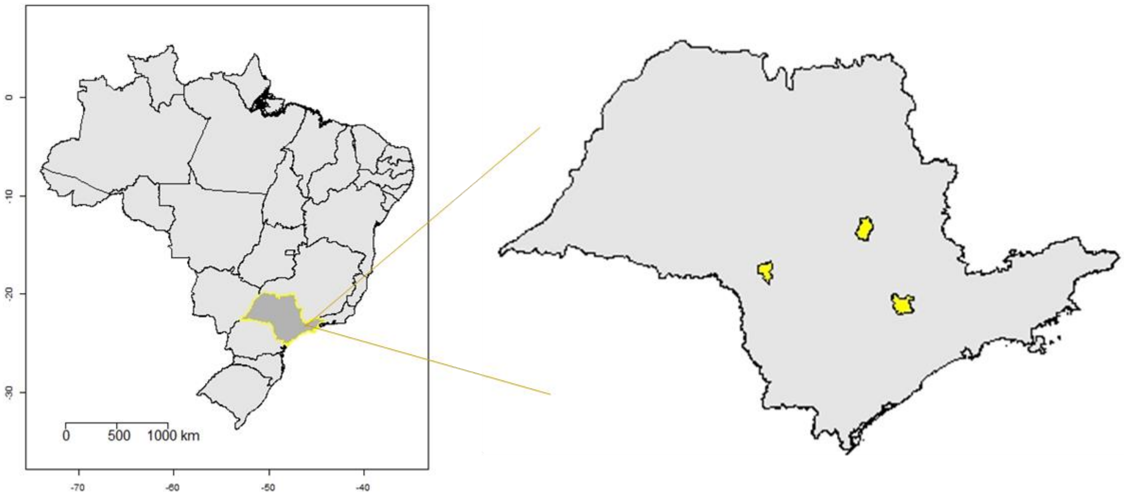

Figura 1. Localização dos municípios em que os estudos foram realizados. Em amarelo estão destacadas nos municípios de Águas de Santa Bárbara, Itirapina e Itu na ordem da esquerda para a direita.

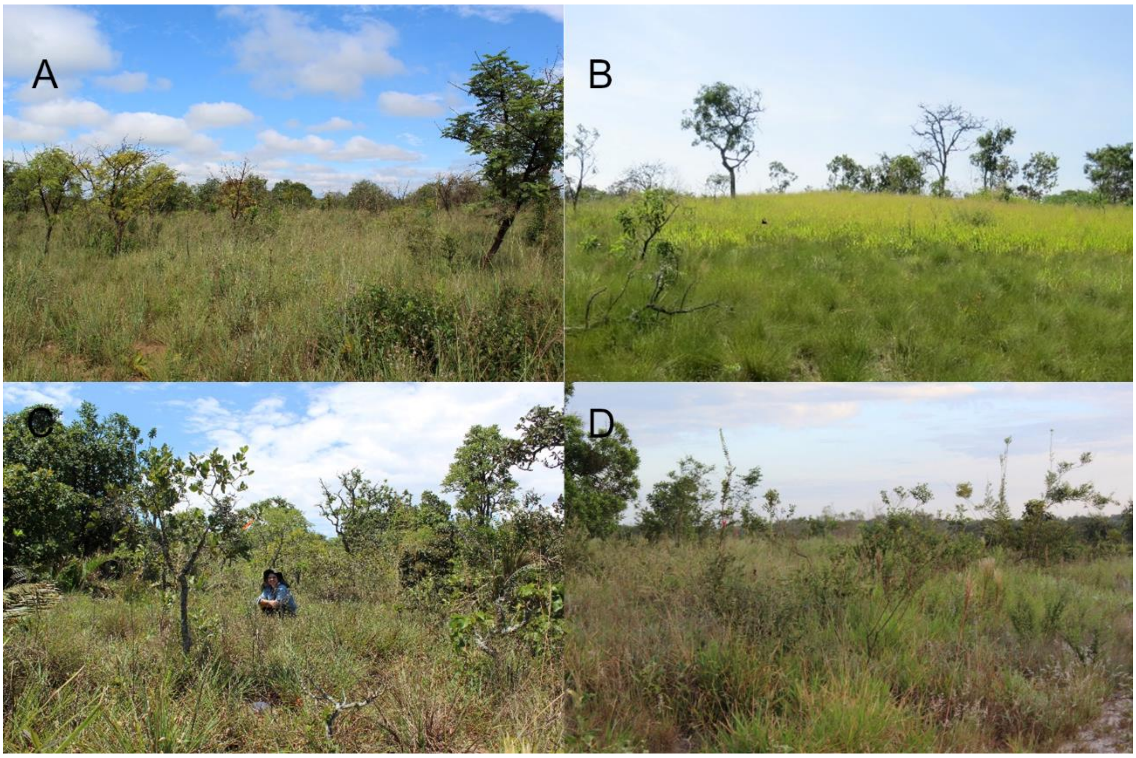

Figura 2. Áreas de estudo: (A) Águas de Santa Bárbara na Estação Ecológica de Santa Bárbara; (B) Primeira área localizada em Itirapina e (C) Segunda área localizada em Itirapina localizadas na Estação Ecológica de Itirapina; (D) Itu, localizada no Condomínio Xapada Itu.

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Capítulo I

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Exotic grass invasion decreases seed predation in a Neotropical savanna

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Abstract

The Neotropical savannas of Central South America (Cerrados) have been invaded by the African grass *Urochloa decumbens* (signal grass or brachiaria), which is replacing native herbs and increasing ground cover. Increased cover can facilitate the foraging by rodents and increase its density due to enhanced protection from predators, but decrease habitat suitability for ants typical from savannas. Both animals frequently interact with seeds. Therefore, changes in ground cover may be accompanied by changes in seed predation. We investigated if the invasion by *U. decumbens* is affecting seed predation rates using seeds from four native plants and the invasive grass. We compared and native and *U. decumbens* seed removal in spots invaded or not by *U. decumbens* using selective exclosures and both seeds buried and above the ground surface in four fragments of Cerrado in southeastern Brazil. Invaded spots had lower seed predation than spots covered by native grasses. Buried seeds had higher survival irrespective of invasion, and interspecific characteristics of each seed species played an important role in removal. Increased seed predation is probably not the cause of decrease native plant regeneration in spots invaded by *U. decumbens*. As invaded spots have modified abiotic conditions that trigger seed germination, such as light, invasion by *U. decumbens* may favour recruitment of shade-germinating species that survive predation.

Keywords: biological invasion, woody encroachment, natural regeneration, invasion mechanism, Cerrado, African grasses

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Introduction

Ants, rodents and birds are important seed removal agents, acting as seed dispersers and/or predators in many ecosystems (Hulme 1998; Vander Wall et al. 2005). Seed dispersal influences the probability of seed survival, germination and transition to seedling stage, increasing the chances of the seed to finding a suitable place for recruitment (Schupp and Fuentes 1995). In contrast, seed predation leads to the eradication of potential individuals in a plant population. Then, microhabitat and seed preferences by granivores may drive plant distribution and community composition (Hulme 1998). Therefore, both seed dispersal and predation are important ecosystem processes influencing plant regeneration and the maintenance of plant community structure and diversity (Christian 2001; Ferreira et al. 2011).

Post-dispersal seed predators can cause considerable losses in different communities (Brown and Heske 1990; Crawley 1992). Rodents and ants are recognized as the most important predators and dispersers of seeds on the ground of Neotropical savannas such as the Cerrados from South America (Hoffmann et al. 1995; Christianini et al. 2007; Briani et al.

2007; Ferreira et al. 2011; Rocha-Ortega et al. 2017), but birds are also responsible for some seed predation (Perez et al. 2006; Rocha-Ortega et al. 2017). Changes in vegetation cover may influence the behavior and abundance of these granivores (Orrock et al. 2010). For example, rodents often prefer areas with higher vegetation cover decreasing their exposure to visually oriented or aerial predators (Manson and Stiles, 1998; Orrock et al. 2004). Some ants may also be affected by changes in shade conditions (King et al. 1998), vegetation structure, and plant species richness (Tews et al. 2004). Decreases in the abundance of ant taxa typical from open savannas have been observed in Cerrados subjected to woody encroachment and increasing shade (Abreu et al. 2017). In addition, some changes in habitat structure (i.e. vertically distributed foliage) may have different impacts on birds, with the effect varying according to bird species (Twes et al. 2004). For example, the convertion native Cerrado to pastures - commonly with the introduction of African grasses such as *U. decumbens* - decreases the occurrence of granivorous bird species typical from Cerrado (Tubelis & Cavalcanti 2000).

Invasive plants often increase the vegetation density (D 'Antonio and Vitousek 1992; Hoffmann and Haridasan 2008; Ferreira et al. 2015), causing changes in habitat structure, microclimate and fire regimes (D 'Antonio and Vitousek 1992; Steenkamp and Chown 1996), as well as the richness, abundance and behavior of granivores (August 1983; Andersen 1986; Dutra and Barnett 2011). Therefore, invasive plants can also affect seed-animal interactions with potential cascading consequences to plant regeneration (Rogers et al. 2017).

The African grass *Urochoa decumbens* (known in Brazil as Braquiária) is well adapted to the climate and soils of Central and Southeastern Brazil where it has been introduced for cattle raising (Boddey et al. 2004). However, this grass has been an aggressive invader and it is spreading throughout the Cerrado (Pivello et al. 1999), a biodiversity-rich Neotropical savanna that once covered 2 million km^2 in Central South America (Myers et al. 2000). The invasion success is assigned to its efficient dispersal and colonization (seeds survive gut passage in cows), fast growth rate, great competitive capacity, tolerance to fire and trampling, and susccessfull establishment after anthropogenic disturbances (Pivello et al. 1999; Gardener et al. 1993). The presence of this invasive grass can impact plant communities, decreasing the survival and recruitment of native seedlings in the Cerrado (Klink 1996; Ferreira 2015; Damasceno et al. 2018). As *U. decumbens* produces a higher biomass than native grasses (Pivello et al. 1999), invaded spots have a lower temperature at ground level, providing invaded locations with higher humidity, lower incidence of sunlight, but increased fire intensity and frequency, compared to non-invaded places (Gorgone-Barbosa et al. 2015). Invasions by *U. decumbens* impact the diversity of bird- (Tubelis & Cavalcanti 2000) and rodent communities (Galvão 2012), but it is uncertain how these changes may be influencing plant-animal interactions and plant regeneration.

The likelihood of seed predation may be influenced by seed size, abundance, presence of reward (fleshy fruit pulp or aril surrounding the seed), chemical composition, and availability to predators (i.e., buried or on the surface). Large and edible seeds are often preferred by granivores (Hulme 1994, 1998), but certain chemicals on the seed or fruit pulp may discourage seed predation (Janzen 1971; Hulme 1998). In addition, seeds that attain a very high abundance in time or space may satiate local seed predators and increase the likelihood of individual seed survival and consequente plant recruitment (Kelly & Sork 2002). *U. decumbens* produces a higher seed-output than native grasses and makes permanent seed banks in soil (Ferreira et al. 2015). Buried seeds may remain undetected by granivores or may be unpreferred due to the higher costs of recovery and handling compared to seeds on the surface (Hulme 1994; Manson and Stiles 1998; Hulme and Borelli 2000). These costs may be relaxed if rodents can recover buried seeds in hidden places, such as spots with high vegetation cover (Manson & Stiles 1998). The seed bank in fire-prone savannas such as the Cerrado is often composed by buried seeds, which represents the regeneration potential of several species in these plant communities (Salazar et al. 2011; Le Stradic et al. 2015). However, the potentital of the invasive *U. decumbens* to change seed predation of buried seeds and change seed bank dynamics in the Cerrado it is still unkown.

In this study we tested: (1) if the invasion by *U. decumbens* changes seed predation of native plants; (2) if buried seeds show higher survival than seeds on the surface; (3) if ants are the most important agents of seed removal irrespective of invaded status of the spot; and (4) if seeds from *U. decumbens* are more likely to survive in spots already invaded by this African grass. We expected that invaded areas had higher seed predation pressure, buried seeds would be more likely to survive, and ants should be the main removal agents in the Cerrado. Finally, seeds from *U. decumbens* would be less removed in invaded sites, due to the high seed availability (e.g. due to predator satiation).

MATERIALS AND METHODS

Study sites

The experiments were conducted in four sites in Southeastern Brazil: Itu (23º17’51.4”S, 47º19’34.1”W), Itirapina 1 (22º14’45.0”S, 47º52’22.3”W), Itirapina 2 (22º12’58.9”S, 47º55’21.7”W) and Águas de Santa Bárbara (22º47’05.7”S, 49º14’29.5”W). Average annual temperatures vary between 18º and 22º. Total rainfall in the driest month is close to 30 mm (June), with annual rainfall varying between 1000 and 1300 mm (SMA 2006; SMA 2011). All study sites are covered mostly by Cerrado, and we choosed the open physiognomies (locally called “campo sujo” and “campo cerrado”) to perform the experiments. We avoided forest physiognomies (Cerradão) since they often present a sparse herbaceous layer and exhibit higher shade than typical savannas. Abundant plants in the study sites included small trees and shrubs such as *Attalea geraensis*, *Ananas ananassoides*, *Byrsonima verbascifolia*, *Campomanesia pubescens*, *Caryocar brasiliensis*, *Miconia rubiginosa* and native grasses such as *Aristida jubata*, *Andropogon bicornis*, *Tristachia leiostachia*, and *Echinolaena inflexa* (SMA 2006; SMA 2011). All sites have several areas invaded by African grasses near areas covered by native grasses, allowing us to set our experiments.

Plant cover

To assess whether Brachiaria invasion affects microhabitat structure, we quantified the percentage of land cover by assigning an index of 1 to 4 for each experimental plot: index 1, corresponding to the presence of 0% to 25% of vegetation covering the quadrant (1mx1m plot); 2 corresponds to 26% to 50%; 3 to 51% to 75%; 4 for coverage of 76% to 100%. Estimates were always made by the same observer (Wikum & Shanholtzer 1978). .

Seed removal

To investigate whether invasion by the African grass *U. decumbens* influence seed removal, we used removal experiments with seeds of the native *Aegiphila verticillata* (hereafter AEG), *Alibertia edulis* (ALI), *Erythroxylum cuneifolium* (ERY), *Myrsine umbellata* (MYR) and the invasive *U. decumbens* (BRA) (Table 1). The four native species were selected due to their occurrence in all study sites and abundant crop sizes, allowing us to harvest the number of seeds needed for the experiments. We used seeds without fruit pulp since our experiments were targeting seed predators. Therefore, we considered that the removed seeds were probably preyed. Since *U. decumbens* often has an abundant seed bank in the soil (Ferreira et al. 2015), we marked the *U. decumbens* seeds used in the experiment with an odorless green paint (Enamel Paint, Testors trademark) to enable us to distinguish experimental seeds from seeds naturally found in seed bank. All seeds were stored in a freezer, to avoid germination, before being used in the experiments.

To avoid confounding effects of seasonality, we performed sampling always in the rainy season (when our seed sources were fruiting) between December and March (summer) from 2017 to 2019. At each site, we established 10 transects 150 m long for sampling (5 transects in non-invaded spots and 5 transects in invaded spots). Transects were at least 80 meters apart from each other. Each transect had 5 plots (1.0 m x 1.0 m) at least 30 m from the nearest one in the transect. Plots established in spots invaded by *U. decumbens* had higher vegetation cover than plots in native grass based on a visual attributed index of cover (F= 13.59; DF = 780; p <0.001) (Fig.1).

Seed removal experiments were performed by using plastic Petri dishes (1 cm high and 7 cm in diameter) divided in half by a 1 cm wall and with several holes for drainage. Each plot received two Petri dishes ca. 30 cm apart from each other and flush with the ground surface: a dish with no exclusion device (open control), and another dish under a metal exclusion cage (1 cm height and 9 cm diameter) staked to the ground and allowing only invertebrate access. Each Petri dish was half filled with local soil over which we added 5 seeds of a plant species on the surface. The other half of the same Petri dish received 5 seeds of the same plant species and soil was added to cover the seed simulating a buried seed bank. Therefore, each Petri dish has two treatments of seed disposition, seeds on surface or buried (at 1 cm) (see Hulme & Borelli, 2000 for a similar approach). In total we used 800 seeds per species in the four study sites (200 seeds per species in each site). The order of seed species arrangement in each plot in a transect was determined at random, but all transects received seeds from all species. The experiment remained in the field for 14 days. After that, we recorded the seeds preyed on *in loco* or removed (seeds not found within a10 cm radius from the Petri dish).

Statistical analysis

Using RStudio environmet (RStudio Team 2016) version 1.1.453, we did a factorial analysis of variance (ANOVA) for comparisons between plant cover in invaded and noninvaded places. To compare seed removal among treatments we performed a generalized linear mixed effects (glmer) model (Poisson family) using the R packages GAD, lme4 and car. Seed disposition (surface or buried), exclusion (with and without cage), seed species and status of invasion (invaded or non-invaded) were inserted as fixed independent factors, and seed removal as the dependent variable. The study site was considered as a random effect. Analyses were done with RStudio environmet (RStudio Team 2016) version 1.1.453.

Results

Vegetation cover differed between native grass and *U. decumbens* (F= 13.59; DF = 780; p <0.001). Plots with the invasive grass presented higher vegetation cover than native grass (Fig. 3). The amount of cover was determinant for seed removal (F = 18.95; DF = 779; p <0.001) (Fig. 2).Contrary to our hypothesis, seeds in native grass plots showed higher predation than those in plots invaded by the African grass (Fig. 3). This pattern was consistent for seed removal in open controls or by invertebrates only (i.e. treatments with the exclusion cage) (Fig. 3). As expected, seed burial decreased seed predation (Table 2; Fig. 4), and therefore buried seeds are more likely to survive both in plots under invasion by *U. decumbens* or in native grassland. If we subtract the amount of seeds removed from caged treatments from open controls, we account that invertebrates were probably responsible for 63% of seed removal while vertebrates would be responsible for the remaining (37%). Therefore, as expected, invertebrates (ants) are the most important agents of seed removal (Fig. 4). Seeds in open controls had higher predation than in caged treatments both on the surface and buried (Fig. 5), but removal rates varied according to the plant species: *Alibertia edulis* and *U. decumbens* showed higher predation than other species, while *Myrcine umbellata* had the lowest levels of seed predation (Table 2; Fig. 6). Interactions between disposition, invasion status (non-invaded or invaded) and plant species were sometimes important for seed predation (Table 2; Fig. 6). We found higher predation rates when seeds of

Alibertia edulis and *U. decumbens* were arranged on the surface and in non-invaded places (Table 2; Fig. 6).

Discussion

Invasion by the African grass *Urochloa decumbens* decreases seed predation by vertebrates and invertebrates in the Cerrado, when considering seeds that were in the soil surface or buried, but the effect differs according to plant species. Since seed predation plays an important role in plant regeneration in many vegetation types, including Cerrado (Ferreira et al. 2011), it is likely that invasion by this African grass may influence the long-term dynamics of Cerrado vegetation under invasion, once that seed predation can decreasing the survival and recruitment of native seedlings (Klink 1996; Ferreira 2015) Indeed, changes in plant communities after invasion by *U. decumbens* and other invasive grasses have been documented for Cerrado (Pivello 1999; Ferreira et al. 2015; Damasceno et al. 2018).

We raised five possible explanations for decreasing seed removal in invaded plots. First, a decrease in plant species richness following invasion (Pivello et al. 1999; Ferreira 2015; Setterfield et al. 2018; Damasceno et al. 2018) may provide a smaller variety of seeds, further decreasing the activity of granivores at invaded spots. Second, *U. decumbens* may change arthropod communities (maybe including granivorous ants) (J. Benitez and A.V. Christianini unpl.data). This is a common result of invasion by exotic plants such as *Prosopis glandulosa* (Steenkamp & Chown 1995) and others (Meijer et al. 2016). Third, tussocks of *U. decumbens* form a bush herb layer compared to native grasses (Fig. 1). This bush microhabitat make the visual detection of seeds on the ground and the locomotion of granivores less efficient (Lassau and Hochuli 2004), decreasing their presence, locomotion and finaly seed removal in invaded spots. Indeed, granivorous birds that forage on the ground level are among the most negativey affected by invasion by African grasses in Cerrado (Tubelis & Cavalcanti 2000). Forth, the invasion modifies the microclimate (De Assis 2017) and this can make these environments unsuitable for seed predators. Finaly, the high seed-output of *U. decumbens* (Xavier et al., 2019) may allow satiation of local seed predators, decreasing the total amount of seeds removed compared to spots with native grasses.

Since *U. decumbens* increases ground cover, the effect of invasion has some resemblances with the increasing shade conditions observed in Cerrado under woody encroachment. Woody encroachment lead to gradual losses of species of plants and ants (the most important source of seed removal in Cerrado) typical from savannas that are replaced often by shade-tolerant and fire sensitive species (Abreu et al. 2017). Changes in rodent community composition in sites invaded by *U. decumbens* have also been observed (Galvão 2012). It is possible that the changes in abiotic conditions influence the fauna and cascade producing a lower seed predation pressure at invaded spots compared to those still covered by native grasses, where cover is lower, more light reaches the soil and temperatures are higher than invaded spots (De Assis 2017).

Our study demonstrates that both native seeds, and *U. decumbens* seeds, incorporated into the soil have reduced predation risks in Cerrado like in other vegetations around the world, thus enabling seed bank formation and species persistence (McDonald et al. 1996). Buried seeds are also likely to have greater chances to survive fire, a natural disturbance in savannas like the Cerrado (Andrade and Miranda 2014). However, the benefit of increased survival for buried seeds contrasts with the relative scarcity of species that establish permanent seed banks in the Cerrado (as the ones used in this experiment, except for *U. decumbens*) (Salazar et al. 2011; Andrade and Miranda, 2014; Le Stradic et al. 2015). Species with a persistent seed bank are known to have lower predation of buried seeds compared to other species (Hulme 2008). Possibly abiotic factors, such as prolonged dry season and high temperatures, coupled with physiological constraints, could account for the lower presence of species in the seed bank of Cerrado rather than predation of buried seeds (Ooi 2012). Invasion by *U. decumbens* has no effect on predation of buried seeds from our focal species. Since spots invaded by *Urochloa* have higher ground cover and humidity, future studies should evaluate if this invasive grass may influence seed bank dynamics by changes in seed survival to pathogenic fungi.

Seed predation varied between species, with *Alibertia edulis* and *Myrsine umbellata* suffering highest and lowest seed predation, respectively. Physical (e.g. seed size, hardness) and chemical (e.g. palatability and presence of secondary compounds) characteristics of seeds usually influence the predator agents (Janzen 1971; Hulme 1994; Perez et al. 2006), and variation in seed predation among different species is common (Hulme 1998; Hulme and Borelli 2000; Ferreira et al. 2011). Seed size does not seem to influence preference by granivores in our study. For example, *U. decumbens* and *A. verticillata* were the most preyed on, although their seeds were similar in size to the species presenting the lowest removal (*Myrsine umbellata*). Since all seeds are relatively small (Table 1), there are probably no constraints to removal even by ants (e.g. plant diaspores up to 1 g are readily removed by ants, see Christianini et al. 2007 and references therein). Seeds from *Myrsine* do not store starch and have xyloglucans (Otegui et al. 1998), which require a special intestinal microbiota for digestion (Flint et al. 2012) (Figure 6), what may explain the lower seed predation in this species.

We found no differences in predation of seeds from the invasive *U. decumbens* in invaded and non-invaded patches (Fig. 5). Therefore, a potentially lower seed predation in invaded sites (for instance due to predator satiation) probably cannot explain the success of this species as an invader. Other traits of *U. decumbens* may be more important for invasion, such as its high seed production, distinct flowering onset and extended flowering period compared to native grasses (see Xavier et al. 2019; D'Antonio and Vitousek 1992; Pivello et al. 1999). There is also a chance that a fraction of the seeds removed by some ants may survive, and thus ants could help to spread *U. decumbens*. This invasive grass is highly competitive for light and water resources which are key for seedling recruitment in Cerrado (Pereira et al. 2009; De Assis et al. 2017). Coupled with low biotic resistance from seed predators, all these traits may increase the success of *U. decumbens* as an invader in Cerrado. The changes in abiotic conditions promoted by this African grass invader, coupled with a decrease in seed predation, may favour the regeneration of shade-tolerant species that are able to deliver seeds to invaded spots, as is the case of *Myrsine umbellata*, which is shade tolerant (Durigan et al. 2004), and presented low predation in invaded Cerrado. *Alibertia edulis* is also tolerant to shading. The ticker cover found at invaded spots decreases light incidence at soil level, which are needed to trigger germination for some plants from Cerrado (Zaidan and Carreira 2008; Salazar et al. 2011; Ferreira 2015), which can decrease the germination and permanence of some species. *Aegiphila lhotzkyana*, for example, is shade intolerant (Durigan et al. 2004). Therefore, even with low predation, its seeds should have a lower chance of recruiting in invaded sites. However, some shade-tolerant plant species are able to colonize Cerrado under woody encroachment (Abreu et al. 2017). In the absence of fire and with decreased seed predation, the first stages of recruitment from shade-tolerant plants may take advantage in invaded spots compared to plants typical from Cerrado. Therefore, it is possible that decreases in seed predation after invasion by *U. decumbens* may interact with fire dynamics and woody encroachment and contribute to long-term changes in vegetation.

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Tables

Table 1. Species used in the seed removal experiments in plots invaded or not by the African grass *Urochloa decumbens* and their characteristics according to Lorenzi (2002). (Zoo = zoochoric, Ane = anemochoric, Intolerant = shade intolerant, Tolerant = shade tolerant)

Scientific name	**Family**	**Growth form**	**Seed length**	**Dispersal Syndrome**	**Shade tolerance**
Myrsine umbellata	Primulaceae	Tree	0.4 cm	Zoo	Tolerant
Erythroxylum cuneifolium	Erythroxylaceae	Shrub	1 cm	Zoo	Tolerant
Urochloa decumbens	Poaceae	Grass	0.4 cm	Zoo/Ane	Tolerant
Alibertia edulis	Rubiaceae	Shrub, tree	0.6 cm	Zoo	Tolerant
Aegiphila verticillata	Verbenaceae	Tree	0.8 cm	Zoo	Intolerant

Myrsine umbellata, *Erythroxylum cuneifolium*, *Alibertia edulis* and *Aegiphila verticillata* are native Cerrado species. *Urochloa decumbens* is na exotic african grass.

Table 2. Table of results from gmler tests evaluating the effects of invasion status (invaded or not), seed disposition (seed buried or on the surface), exclusion (seeds available under an exclusion cage or in an open control), seed species used in the experiments, and their interactions on seed removal. Since our model allowed many interactions, we added to the table only those treatments that were significant (p <0.05). Acronyms: *Aegiphila verticillata* (AEG), *Alibertia edulis* (ALI), *Erythroxylum cuneifolium* (ERY), *Myrsine umbellata* (MYR) and the invasive African grass (*Urochloa decumbens*) (BRA)

	Estimate	z value	p
(intercept)	-0.884	-3.612	<0.001
Nativegrass	0.982	3.832	<0.001
SurfaceDisposition	1.348	5.377	<0.001
WithoutExclusion	0.401	4.356	<0.001
SpeciesAlibertia	1.272	5.161	<0.001
SpeciesUrochloa	0.944	3.668	<0.001
SpeciesErythroxylum	0.592	2.182	0.029
Nativegrass:SurfaceDisposition	-0.883	-3.012	0.002
Surfacedisposition:WithoutExclusion	0.239	2.141	0.032
Nativegrass:SpecieAlibertia	-0.870	-2.878	0.003
Nativegrass:SpecieUrochloa	-1.073	-3.294	<0.001
SurfaceDisposition:SpecieAlibertia	-0.964	-3.424	<0.001
SurfaceDisposition:SpecieUrochloa	-0.798	-2.719	0.006
Nativegrass: SurfaceDisposition:SpecieAlibertia	1.004	2.828	0.004
Nativegrass: SurfaceDisposition:SpecieUrochloa	1.212	3.197	0.001

Figures

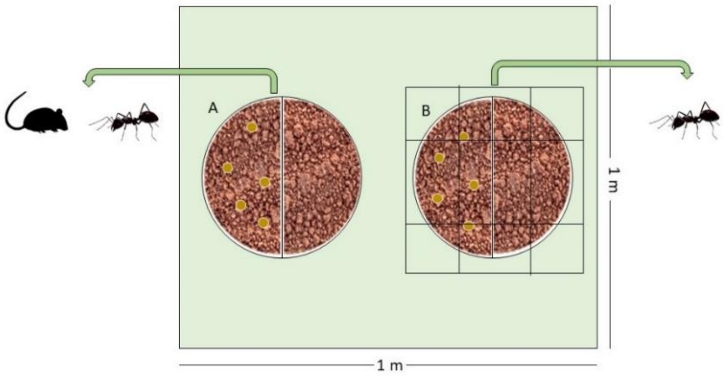

Fig 1. Diagram showing the layout of the field experiment in plots (1m x 1m). Petri dishes were divided with five seeds arranged on the surface and five buried seeds. (A) Experiment without exclusion cage (control) allowing access by rodents and ants; (B) Exclusion cage experiment allowing only ants access.

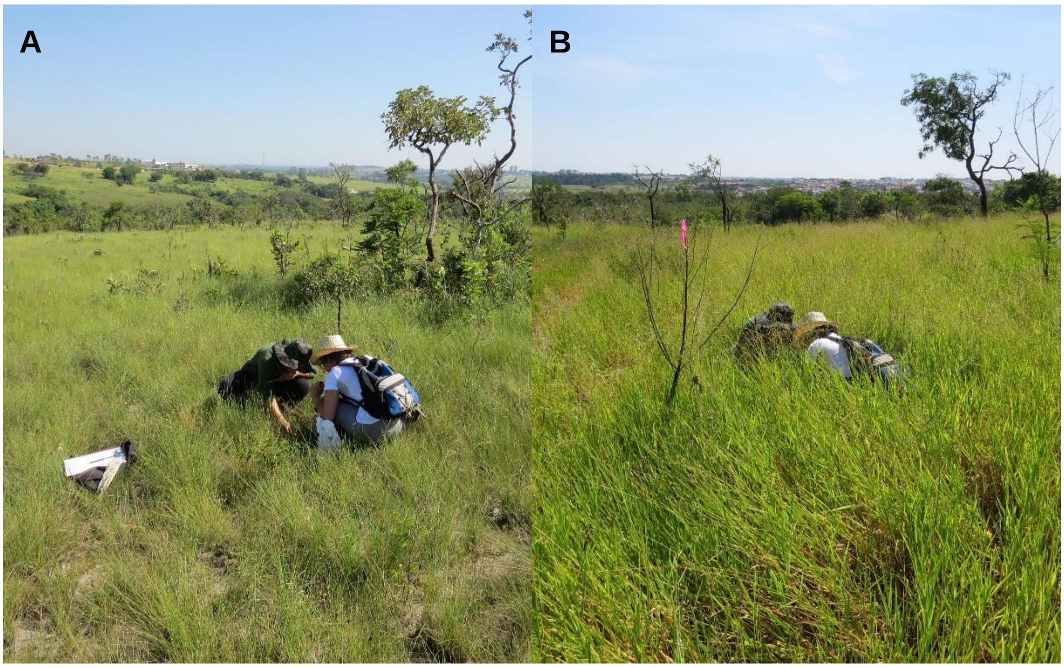

Fig 2. Contrast of ground cover between: (A) Non-invaded places that have less dense foliage and spacing between tickets, and (B) Invaded places by *Urochloa decumbens* that have denser foliage and smaller spacing between trickets. Photographic record made at de Santa Barbara Ecological Station - SP. (personal archive)

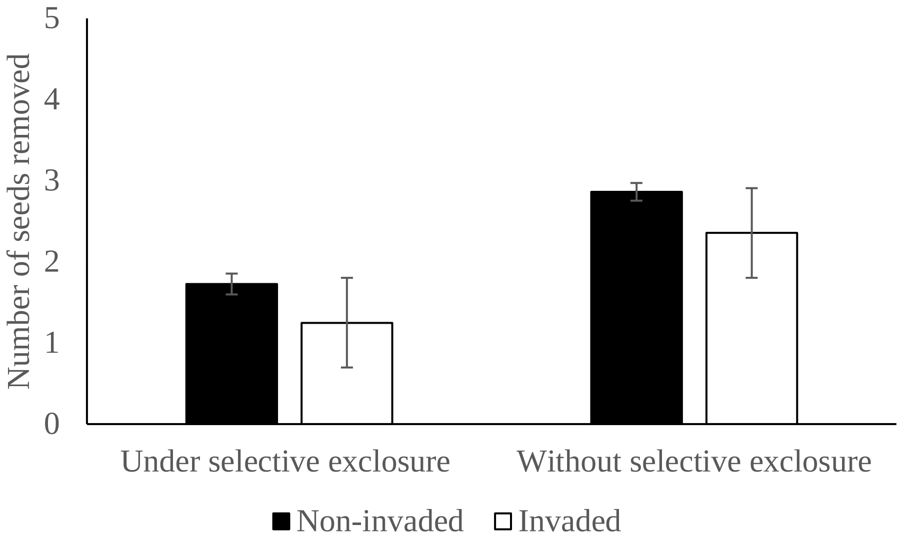

Fig 3. Average ± SD seed removal by invertebrates (under selective exclosure cage) and both vertebrates and invertebrates (without selective exclosure) in invaded (presence of *Urochloa decumbens*) and not invaded spots. Data for all sites combined.

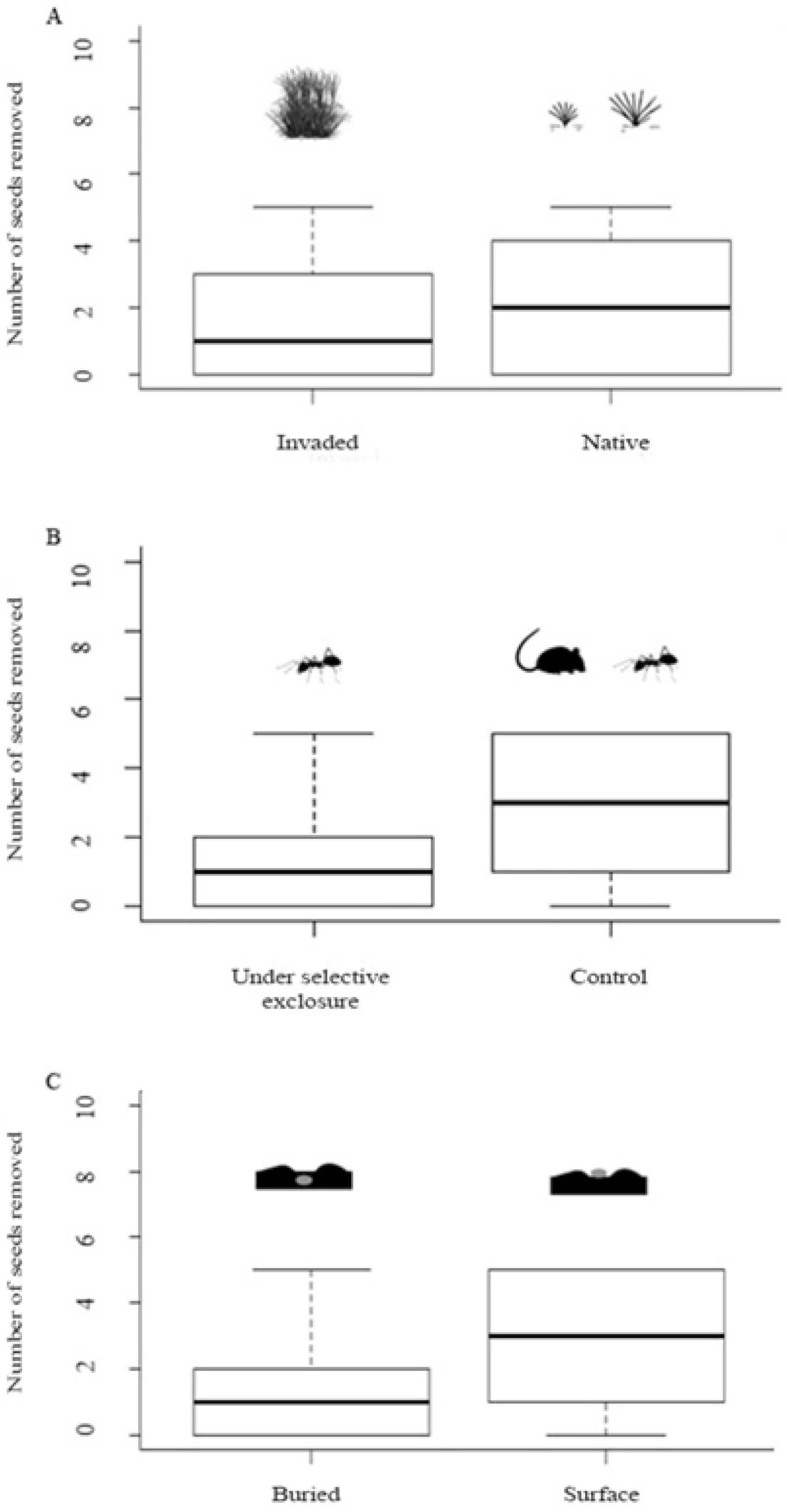

Fig 4. Seed removal in: (A) spots invaded by *Urochloa decumbens* and not invaded; (B) under selective exclosure or controls; (C) buried or on surface. Data for all plots combined (N = 400).

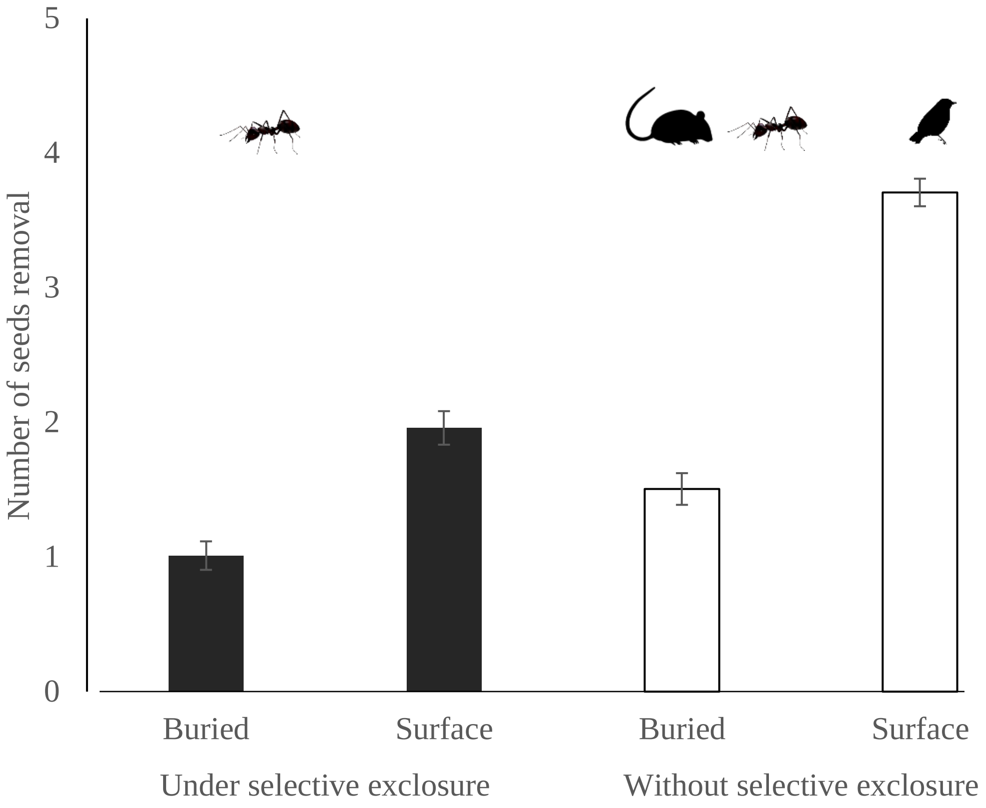

Fig 5. Average values ± SD of seed removal (all five seed species) according to the presence or absence (control) of exclusion cage (N=400 each), and the disposition of seed buried (N=200) or on surface (N=200) in four sites of Cerrado in southeastern Brazil.

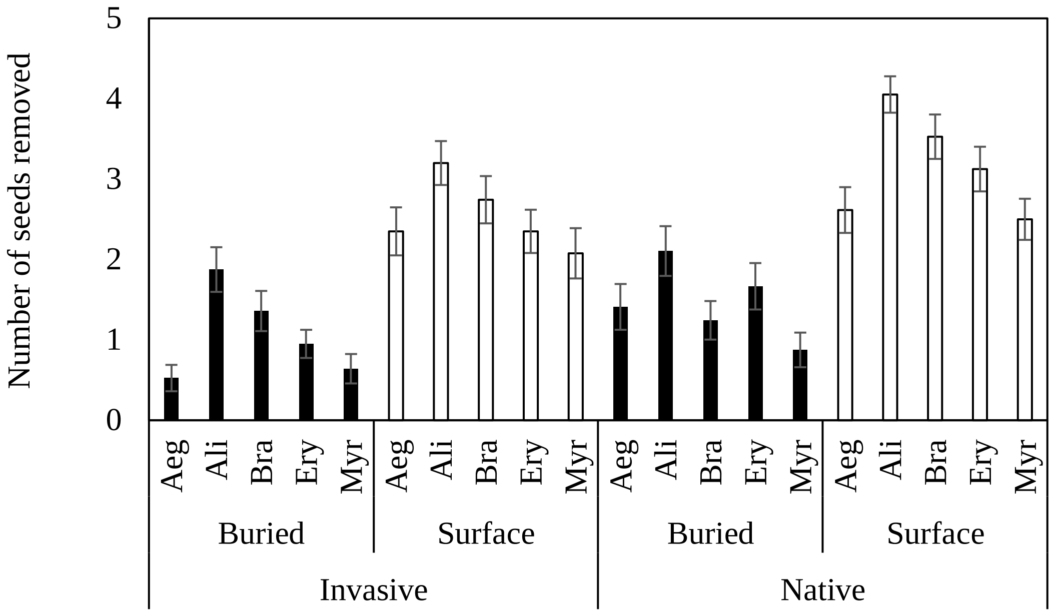

Fig 6. Average values ± SD of seed removal in four sites in southeastern Brazil according to type of grass covering plot (invasive or native), disposition (buried or in surface) and species used in the experiment. *Aegiphila verticillata* (AEG), *Alibertia edulis* (ALI), *Erythroxylum cuneifolium* (ERY), *Myrsine umbellata* (MYR) and the exotic invasive grass (*Urochloa decumbens*) (BRA). Data for all sites combined.

Capítulo II

Insight escrito nas normas do periódico Biotropica.

BIOTROPICA
THE JOURNAL OF THE ASSOCIATION FOR TROPICAL BIOLOGY AND CONSERVATION

LRH: Rebolo *et al.*

RRH: Native ants spread an invasive grass

Native ants decreases the spread an invasive African grass in the Cerrado

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ABSTRACT

Plant-animal interactions may facilitate biological invasions. The African grass *Urochloa decumbens* is an aggressive invader in the Brazilian Cerrado. We demonstrate that native ants are dispersing the seeds to short distances, allowing the gradual expansion of the invasive to sites without the need of great anthropogenic soil disturbances.

Keywords: biological invasion, biotic resistance, *Brachiaria*, disturbance, savanna, seed dispersal, *Urochloa*, woody encroachment

Invasive plants often change the microhabitats and microclimates, fire regimes, nutrient cycles and replace native species in invaded sites (D' Antonio & Vitousek 1992, Mack et al. 2000, Vilá et al. 2011). Invasive success usually relies on a better performance of the invader compared to natives due to, for example, release from natural enemies and competitors (Levine et al. 2004), and fluctuations in resources that usually follows disturbances and that coincides with availability of invasive species propagules (Davis et al. 2000). Establishment of successful mutualisms with native animals in the invaded ecosystems, such as pollination and seed dispersal, is also key to invasive success (Richardson et al. 2000). It is well known that seed dispersal is important for patch colonization of plants, and thus, a better knowledge about dispersal of propagules is crucial to manage and control invasive species (Puth & Post 2005).

Signalgrass *Urochloa decumbens* (Stapf) R.D. Webster (syn. *Brachiaria decumbens* Stapf) is a perennial C4 African grass introduced in Brazil for cattle ranching in 50's (Miles et al. 1996). This grass became popular among ranchers and now ca. 45% of the Cerrado domain (ca. 500.000 km²) is occupied by pastures planted with *U. decumbens* and other African grasses (Klink e Machado 2005). *Urochloa* spp. are aggressive invaders in tropical savannas and grasslands of the Cerrado, including most of the protected areas (Pivello et al. 1999a, 1999b, Durigan et al. 2007). These African grasses change shade conditions, fire regimes (Gorgone-Barbosa et al. 2015), and outcompete native species, mostly native grasses (Pivello et al. 1999a, 1999b, Damasceno et al. 2018), since they have alelopathic compounds (Barbosa et al. 2008) and a high production of biomass (Damasceno et al., 2018), threatening this global biodiversity hotspot (Myers et al. 2000). *U. decumbens* has a high seed output (Xavier et al. 2019) and fast colonization of disturbed patches, such as spots of soil disturbed by plowing. Indeed, invasion of Cerrado by African grasses often starts with the colonization of disturbed soil patches, such as the edge of roads and firebreaks (Pivello et al. 1999a, 1999b), and soil dumps (*murundus*) formed by the excavation of leaf-cutter ant nests (Coutinho 1982). In these patches, temperature fluctuation is higher what increases the percentage and speed of *Urochloa* seed germination (Gorgone-Barbosa et al. 2016). There is a common perception that without further vegetation and soil disturbances, *Urochloa* is unlikely to penetrate deep into Cerrado, being more restricted to the disturbed edges of remnants. However, vegetation samplings from edges to the core areas of remnants show that this is not always the case (Pivello et al. 1999a, Dodonov et al. 2019).

The local spread of *U. decumbens* may happens by vegetative reproduction, but long distance dispersal relies probably on seed dispersal by wind (Assis 2017, Xavier et al. 2019), traffic of vehicles and cattle (Pivello et al. 1999a, Veldman & Putz 2010). Seeds of *U. decumbens* have a low survival probability, because rodents, birds and ants feed on the seeds and may provide some biotic resistance against invasion (I. F. Rebolo and A.V. Christianini, unpl. data). However, seeds from *U. decumbens* can also form transient seed banks (Dantas-Júnior et al. 2018), with a high seed density in the soil seed bank in both invaded and in neighboring non-invaded sites (Dairel & Fidelis, submitted). Ants in particular may be important, because they interact with many plant diaspores on the ground of Cerrado, constraining the recruitment of several native plants (Ferreira et al. 2011). However, ants can sometimes disperse a fraction of seeds they interact, even those not adapted for ant dispersal (i.e. non-myrmecochorous) (Christianini et al. 2007). Granivorous ants often discard intact seeds around nest entrances, for instance due to seasonal fluctuations in the availability of preferred seeds or other food resources (Rissing 1986, MacMahon et al. 2000). These seeds dispersed to ant nests may have a higher likelihood of recruitment, because the ant nest usually provide better soil conditions (Farji-Brener & Werenkraut 2015) or protection against insect herbivores (Passos & Oliveira 2004). During a walk in Cerrado it is not uncommon to find seeds of *U. decumbens* accumulated around ant nest entrances (author's pers. obs.; Figure 2). If those seeds are still viable, ants may be contributing to the dispersal of invasive grasses.

We evaluated the potential of seed dispersal of the invasive African grass *Urochloa decumbens* by ants in a Cerrado fragment of 150 ha at Estação Experimental de Itirapina (22°12'S, 47°51'W), southeastern Brazil, between February and March 2019 when *U. decumbens* was releasing its seeds. Local mean annual rainfall is 1,360 mm, concentrated in the hot and wet season (December to March), and mean annual temperature is 21.8ºC. The study site is covered by dense savanna (Cerrado), divided by several firebreaks about 20 m width. Firebreaks have patches of soil covered by native (e.g. *Gymnopogon foliosus*) and exotic African grasses (e.g. *U. decumbens*, *Melinis minutiflora*). Like in other areas of Cerrado, it is common to find seeds of *U. decumbens* around nest entrances (Figure1).

To investigate if ants may disperse the seeds of *U. decumbens*, we sampled ant nests found in each of three 346 m x 1 m transects placed at edges or in the center of the firebreaks. The transects were sampled by the same person (I.F. Rebolo) walking slowly (< 1 km.$^{h-1}$) and looking for concentrations of seeds around ant nests or ants carrying seeds of *U. decumbens*.

When an ant nest with seeds was found, the nest was marked and a sample of ants collected for further identification (Figure 1). Our method was not designed to provide an exhaustive list of ants interacting with *U. decumbens* or to sample all ant nests in each transect, but to sample the most active and conspicuous ant nests with seeds of the invasive. To estimate minimum distances of seed removal by ants we recorded the distance from ant nest up to the nearest tussock of *U. decumbens* in reproduction. To investigate the role of ants as seed predators or dispersers we established a sample plot of 10 x 10 cm centered at the nest entrance and harvested all seeds of *U. decumbens* at soil surface. To record seed availability, we also sampled seeds found in four 10 x 10 cm plots established in paired-controls 1 m away in North, South, East and West direction from the ant nest (Figure 1). All seeds were taken to the lab, counted and sorted under a stereomicroscope as non-damaged, damaged or empty, and further stored at room temperature. To increase independence of observations, we sampled seeds only from ant nests at least 10 m away from another sampled ant nest.

We obtained a density of 0.03 ant nests.m^{-2} (ca. 318 ant nests.hectare), with 22, 8 and 3 ant nests sampled at each transect. We sampled seeds of *U. decumbens* from 21 ant nests. Estimates of seed removal varied from 15 to 120 cm (median = 40 cm). All ant nests with seeds were from the fungus growing ant *Mycocepurus goeldii* (Attini). Plots in ant nests had the median number of seeds (16; range = 3-364) around five times higher than control plots (3.3; range 0-33) (Wilcoxon paired-sample test: W=231; p<0.001). We found more absolute numbers of non-damaged (W=253; p<0.001), damaged (W=325; p<0.001) and empty seeds (W=820; p<0.001) around ant nest entrances than at control means (Figure 3). The percentage of non-damaged seeds in ant nests (median=0%; range 0-33.3%) was similar to that from controls (6.9%; 0-62.5%; W=35; p=0.052), as well as damaged seeds (ant nests: 2.7%; 0-66.7%; controls 5.9%; 0-100%; W=58; p=0.623). However, ant nests had a higher proportion of empty seeds (92.3%; 25-100%) compared to controls (77.8%; 0-100%; W=181; p=0.022).

We tested the viability of non-damaged seeds using two approaches. First we performed germination experiments. Seeds were placed in Petri dishes using filter paper regularly wet with distilled water and kept at constant light at 25 ºC. Seed germination was checked twice a week, removing any germinated seed thereafter. After 30 days we tested the viability of the remaining non-germinated seeds with a solution of Tetrazolium (Brasil 1992). Seeds were cut and immersed in a 1% Tetrazolium solution in a Petri dish, involved with aluminum foil and kept at dark at 35ºC for 24 hs. After that, seeds were inspected under a stereomicroscope and if the embryo was stained reddish, seeds were considered viable. We found 50% more viable seeds around ant nest entrances than at controls away (Wilcoxon paired-sample test: W= 231; p<0.001; Figure 3).

Seeds from exotic plants are often included in the menu of native granivorous ants when available (Aput et al. 2019). The fungus-growing ant *Mycocepurus goeldii* harvest a diversity of items including seeds, leaf fragments, insect frass and pulp of fleshy fruits fallen to the ground to cultivate their fungus inside ant nests (Leal & Oliveira 2000). Seeds from grasses are often richer in carbohydrates and have less secondary compounds than seeds from trees (Ríos et al. 2012), what probably benefit the growth and fitness of the fungus inside *M. goeldii* nests. *M. goeldii* is also reported exploiting seeds of *Urochloa* sp. in another study (Christianini et al. 2012).

The higher density of seeds around the entrance of nests of *M. goeldii* than in controls indicates that this ant is actively removing seeds from other sources away. The higher proportion of empty seeds may be due to an accumulation of seeds discarded by ants after removing the seed husk and taking the soft tissues to the fungus chambers, preying on these seeds. *U. decumbens* also produces large numbers of empty seeds due to failure in pollination (Mendes-Bonato et al. 2001). The higher number of viable seeds of *U. decumbens* around ant nests than in controls indicates that *M. goeldii* is actively dispersing seeds of this invasive African grass, contributing to its invasion success.

Fungus-growing ants often discard some items brought to their nests (delayed rejection, Arenas & Roces 2017), what may increase the chance that these ants disperse seeds to waste dumps (at surface in the case of *M. goeldii*). Distances of seed removal (from 15 to 120 cm) are compatible to foraging distances of *M. goeldii* recorded in other localities of Cerrado (129 ± 98 cm, mean ± SD, Leal & Oliveira 2000). Therefore, we believe that ants are helping to spread the invasive grass at short distances, expanding gradually the wave of invasion toward uninvaded Cerrado. Actually, non-invaded neighboring areas in the Cerrado already have their seed bank with seeds of *Urochloa* spp. (Dairel & Fidelis, submitted). This scale of spread is compatible with reports of the dissemination of invasive grass and forb species around the world (Pyšek & Hulme 2005). Indeed, the high density of nests (a nest at each 32 m^2 in our sampling) and the large number of seeds at nests indicate that interactions between *M. goeldii* and seeds of *U. decumbens* are common. Although we never recorded leaf-cutter ants (*Atta sexdens* and *A. laevigatta*) interacting with seeds of *U. decumbens*, these ants may play a role in the expansion of the invasive grass. These ants prune the vegetation around the ant nest and deposit large amounts of excavated soil at the surface creating patches free of vegetation that are hotspots for the colonization of invasive African grasses (Coutinho 1982). Thus, gradual spread of seeds by *M. goeldii*, combined with long-distance colonization of leaf-cutter ant nests by seeds dispersed by wind or other means may allow the invasion into Cerrado fragments without the need of any further anthropogenic disturbance. Ant nests may also be very good microsites for early establishment of exotic plants (Farji-Brener et al. 2010). Thus, *M. goeldii* is probably not only providing an inefficient biotic resistance against invasion, but it is also providing seed dispersal and potential better conditions for the establishment of *Urochloa*. The good news is that dispersal by *M. goeldii* happens at short distances. The bad news is that this ant is quite abundant and may be dispersing seeds without notice, helping to spread the invasive step by step into more conserved Cerrado.

FIGURE LEGENDS

Figure 1. View of the immediate surroundings of a nest of the ant *Mycocepurus goeldii* in the Cerrado (southeastern Brazil). Many seeds of the invasive African grass *Urochloa decumbens* are found around nest entrance (arrow).

Figure 2. *Mycocepurus goeldii* ants concentrate seeds of the invasive African grass *Urochloa decumbens* around ant nest entrances in Cerrado from southeastern Brazil. (a) Number of non-damaged, damaged and empty seeds sampled at 100 cm^2 quadrats at controls and ant nests; (b) Proportion of viable seeds found at controls and ant nest entrances (considering only non-damaged seeds). All values are mean ± SE.

Figure 3. Schematic representation of the layout of the field experiments. The ant nest entrance is located in the center, where *U. decumbens* seeds were sampled within the 10 cm x 10 cm plot. For control, seeds were sampled at 1 meter to the North, South, East and West within 10 cm x 10 cm plots.

Figures

Figure 1.

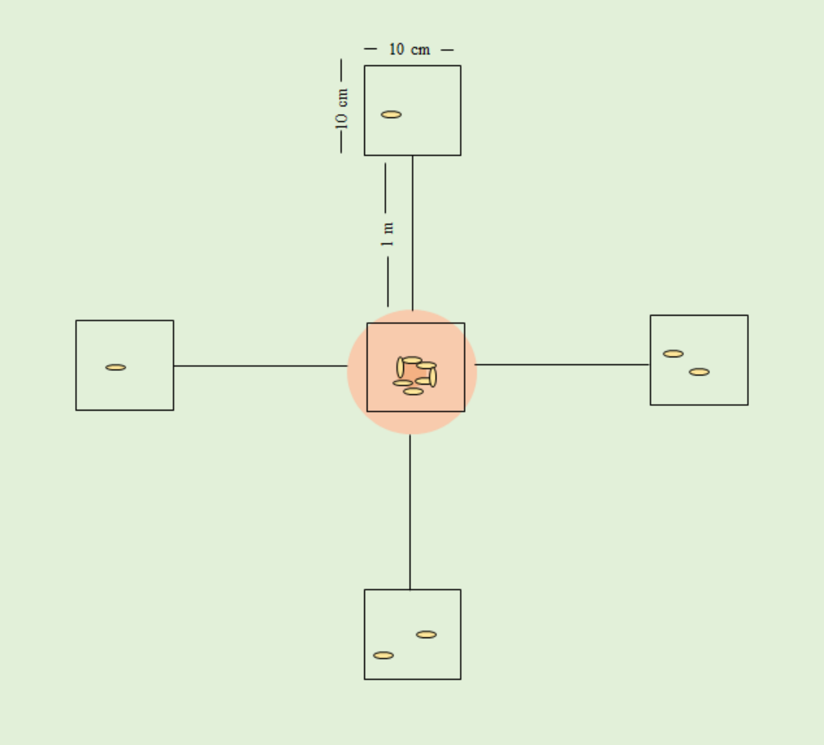
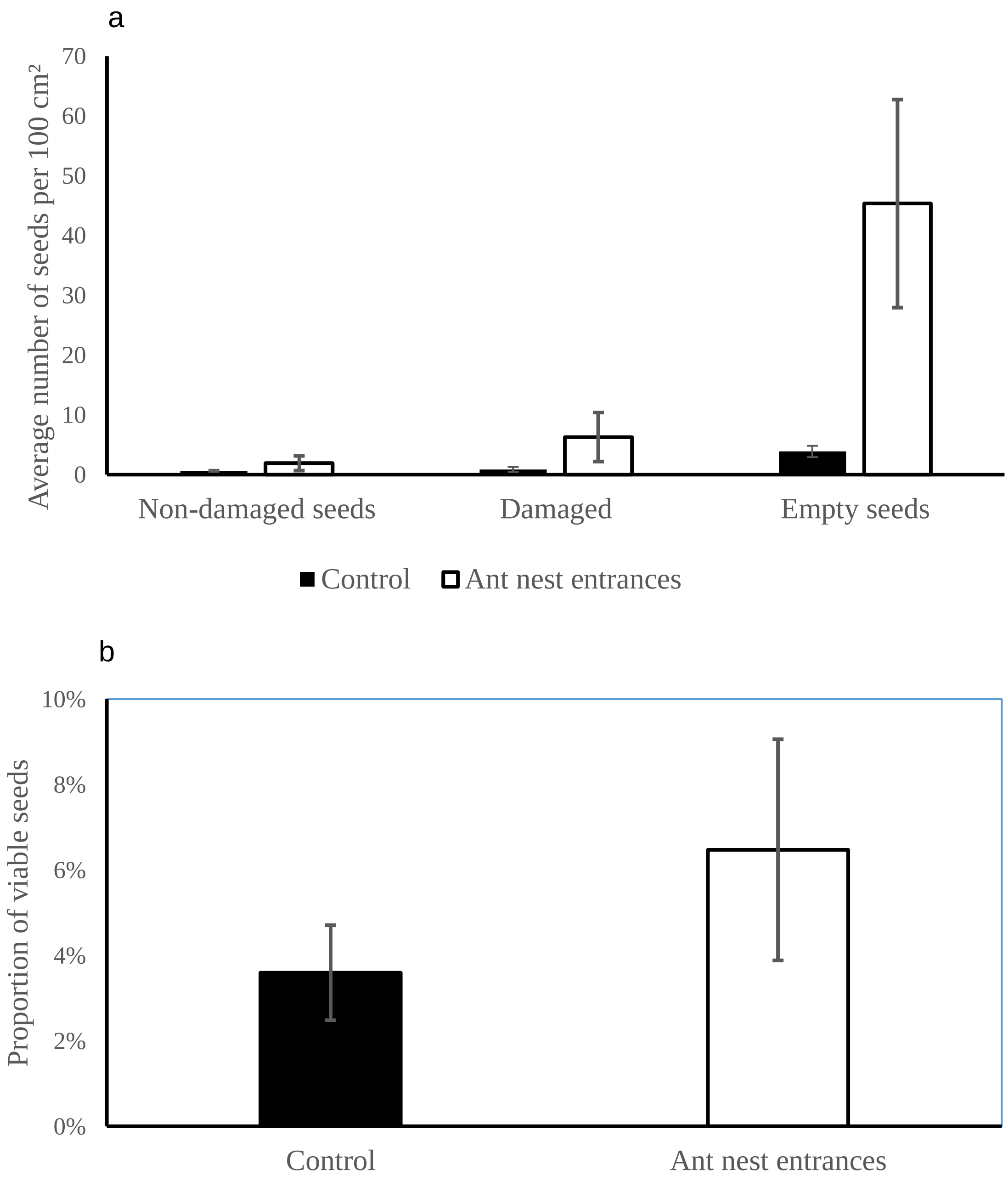


Figure 2

Figure 3


a
Average number of seeds per 100 cm²
70
60
50
40
30
20
10
0
Non-damaged seeds
Damaged
Empty seeds
■ Control
□ Ant nest entrances
b
Proportion of viable seeds
10%
8%
6%
4%
2%
0%
Control
Ant nest entrances

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DISCLOSURE STATEMENTS

The authors declare no potential conflict of interests

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SUPPORTING INFORMATION

APPENDIX S1: Abstract in Portuguese (Resumo)

Interações planta-animal podem facilitar invasões biológicas. A gramínea *africana Urochloa decumbens* é uma invasora agressiva no Cerrado Brasileiro. Nós demonstramos que formigas

nativas estão dispersando as sementes a curtas distâncias, permitindo a expansão gradual da invasora para sítios sem a necessidade de grandes perturbações do solo pelo homem.

Considerações Finais

A presença de *Urochloa decumbens* parece estar modificando a dinâmica de algumas interações entre plantas e animais no Cerrado. Fatores como características intraespecíficas da espécie, disposição da semente (se enterrada ou na superfície) e locais invadidos interferem nas taxas de predação de sementes. Sementes quando enterradas aumentam as chances de sobrevivência, indicando um potencial para contribuir na regeneração das plantas a partir de sementes incorporadas ao banco de sementes. A predação de sementes parece não explicar a dificuldade de regeneração de espécies nativas em locais invadidos. Portanto, as mudanças na composição florística e diversidade de plantas em locais invadidos estão provavelmente ligados a outras etapas, por exemplo envolvendo dificuldade na germinação e sobrevivência das plântulas, talvez devido às mudanças microclimáticas e competição com *U. decumbens*. Estas mudanças proporcionadas pela invasão, como o sombreamento, apresentam potencial para modificar o perfil de plantas que recrutam em locais invadidos, podendo influenciar – talvez - no adensamento da vegetação do Cerrado. Vimos que a dispersão de *U. decumbens* parece estar sendo potencializada pela ação de formigas nativas, como *Mycocepurus goeldii*. Desta maneira a disseminação da invasora pode ocorrer sem a presença de perturbações antrópicas, tornando o controle da invasora ainda mais difícil.