



**UNIVERSIDADE ESTADUAL PAULISTA**  
**“JÚLIO DE MESQUITA FILHO”**  
**INSTITUTO DE BIOCÊNCIAS - RIO CLARO**



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

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**A vegetação campestre em gradientes  
inundáveis: composição florística, dinâmica  
do banco de sementes e de plântulas**

**Francielli Bao**

**Outubro - 2017**

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BIOLOGIA VEGETAL

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# A vegetação campestre em gradientes inundáveis: composição florística, dinâmica do banco de sementes e de plântulas

**Francielli Bao**

Tese apresentada ao Instituto de Biociências do Campus de Rio Claro, Universidade Estadual Paulista, como parte dos requisitos para obtenção do título de Doutor em Ciências Biológicas (Biologia Vegetal).

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**Outubro - 2017**

582.0467 Bao, Francieli

B221v A vegetação campestre em gradientes inundáveis:  
composição florística, dinâmica do banco de sementes e de  
plântulas / Francieli Bao. - Rio Claro, 2017  
169 f. : il., figs., gráfs., tabs.

Tese (doutorado) - Universidade Estadual Paulista,  
Instituto de Biociências de Rio Claro  
Orientador: Marco Antônio de Assis  
Coorientador: Arnildo Pott

1. Sementes. 2. Espécies exóticas. 3. Inundação. 4.  
Invasão de espécies. 5. Áreas úmidas. 6. Banco de sementes.  
7. Controle de espécies invasoras. I. Título.

**CERTIFICADO DE APROVAÇÃO**

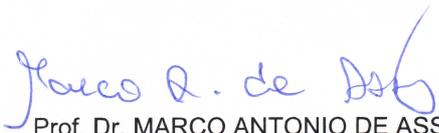
TÍTULO DA TESE: A vegetação campestre em gradientes inundáveis: composição florística, dinâmica do banco de sementes e de plântulas

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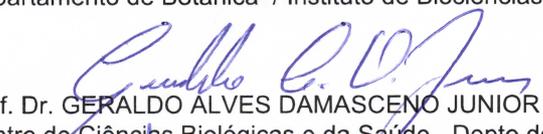
**COORIENTADOR: ARNILDO POTT**

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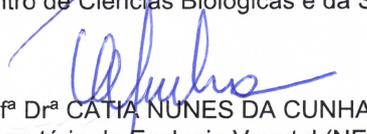
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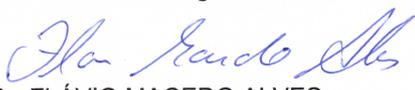
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Rio Claro, 10 de outubro de 2017

A família Bao, Ires e Zelindo,  
Cristina, Giovana e Laura, dedico com  
amor esta tese.



"Ao Pantanal deixo minha gratidão, lá não fui encontrar apenas um lugar para pesquisa, sinto que foi ele quem me chamou para encontrar algo que dentro de mim, há algum tempo havia perdido".

## Agradecimentos

Ao Programa de Biologia Vegetal, do Instituto de Biociências da Universidade Estadual Paulista, de Rio Claro, pela oportunidade de realização do doutorado.

Ao Programa de Biologia Vegetal, do Centro de Ciências Biológicas da Universidade Federal de Mato Grosso do Sul, pelo apoio e infraestrutura no projeto.

Ao INAU - Instituto Nacional de Ciência e Tecnologia de Áreas Úmidas, pelo apoio financeiro para realização deste projeto.

Ao Dr. Marco Antonio de Assis, pela orientação e confiança, principalmente por ter aceitado o projeto mesmo com a realização, deste, no Pantanal.

Ao Dr. Arnildo Pott, por ter-me apresentado ao Pantanal e ao pequeno, porém grande universo das sementes, obrigada pelo aprendizado em sua orientação nestes anos.

Ao Dr. Rafael Arruda, por tantas conversas científicas nestes anos de pós-graduação.

À Dra. Tracy Elsey-Quirk, pelas sugestões e revisões dos trabalhos, sempre com comentários que engrandeceram este projeto.

À Dra. Alessandra Tomaselli Fidelis, que revisou todos os relatórios e sempre nos deixou comentários e sugestões que contribuíram na redação dos capítulos.

À Dra. Alessandra Ike Coan pela ajuda e atenção com minhas necessidades burocráticas junto à pós-graduação, quando mais precisei.

Ao CNPq - Conselho Nacional de Desenvolvimento Científico e Tecnológico (Processo - 162248/2013-0) pela concessão de bolsa durante o período do doutorado.

À Fazenda São Bento, em nome do gerente Célio, obrigada por nos possibilitarem a realização do projeto desde o mestrado.

À Deborah Bambil, pela amizade e companheirismo, pela ajuda na realização de todas as coletas, elaboração e monitoramento dos experimentos. Obrigada pela amizade dentro e fora da universidade, por tantas conversas e apoio, pois ninguém faz nada sozinho, obrigada por sua alegria contagiante.

Ao Dr. Thales D. Leandro, não somente pela amizade nestes anos, mas também por toda ajuda burocrática junto à pós, sempre que precisei.

Aos amigos do laboratório de Ecologia Vegetal da UFMS, pelas conversas diárias, Anny, Darlene, Diego, Evaldo e Rosa, obrigada.

Aos amigos do Herbário CGMS, pela amizade, conversas e cafés, Ana, Camila, Fábria e Thiago, obrigada.

À Dra. Gisele Catian pela ajuda na luta com a identificação de sementes.

As grandes amizades que fiz em Campo Grande, que tornaram meus anos nesta cidade tão especiais, desde o pensionato (a família Green - Zé e Raquel, meus pais campograndenses, Mari e Tiago, Dani, Micheli, Paty, Pri e Zita). Ao time de basquete de Mato Grosso do Sul, que tive a oportunidade de integrar e conquistar tantos títulos nestes anos (Ronaldo, técnico e amigo querido, Bruna, Camila B, Camila T, Carla, Flavia, Suy e não menos importantes, mas todas do time UCDB e time Máster).

As grandes amigas Carol e Karen que aguentaram meus momentos de loucura e sumiço nos treinos, amigas do basket que levarei para vida, foram tantas as conversas, desabafos, cervejas e risadas, obrigada pelo apoio e compreensão nestes anos.

À Grazi, amiga-irmã que escuta, apoia e compartilha todas as minhas/nossas "loucas" ideias, sonhos e objetivos. É bom ter você por perto.

À minha família, pai, mãe e mana e, sobrinhas (gigi e Laurinha) que sempre entenderam e apoiaram meus sonhos e vontades, à distância nestes anos não foi fácil, mas o apoio de vocês fez tudo se tornar mais simples e fez essa distância parecer tão pequena. A importância que toda a pós-graduação teve em si em minha vida vocês sabem e, graças a esse apoio que vive os melhores anos da minha vida (até aqui)! Amo vocês!

A Deus, que jamais vou cansar de agradecer, por guiar meus passos e estes passos terem me trazido até aqui, não poderia imaginar profissão melhor, lugar melhor ou vida melhor da que tenho hoje!

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## Resumo

Espécies exóticas como *Urochloa humidicola* (braquiária) são introduzidas em pastagens nativas e ameaçam a diversidade de áreas úmidas, como o Pantanal. Em áreas úmidas submetidas a eventos extremos sazonais, a reserva de sementes no solo é uma importante fonte de regeneração da vegetação. Informações sobre a influência de plantas exóticas e da inundação com relação à dinâmica do banco de sementes, distribuição e composição da vegetação campestre ajudam a elucidar as características adaptativas voltadas para germinação, crescimento e estabelecimento das espécies. O objetivo foi avaliar as fontes primárias de regeneração: o banco de sementes e de plântulas, sob influência de diferentes níveis de topografia e sazonalidade e, estabelecimento de plantas exóticas. As coletas ocorreram em dois campos de pastagem, um dominado por espécies nativas e outro dominado por *U.humidicola*, no Pantanal de Mato Grosso do Sul. A amostragem ocorreu em lagoas sazonais onde foram estabelecidos três níveis topográficos (baixo, intermediário e alto), além disso, para analisar o efeito da sazonalidade, as coletas foram realizadas ao final das estações de seca e de cheia, em dois anos. Os dados da vegetação, do banco de plântulas e de sementes foram analisados separadamente, onde foi possível avaliar cada estágio de vida em função da sazonalidade, topografia e influência de plantas exóticas. Em seguida os dados de vegetação e banco de sementes foram relacionados para verificarmos a importância do banco de sementes na manutenção da flora nativa. Sendo assim, o primeiro capítulo abordou a influência da *U.humidicola* sobre a pastagem nativa e, o papel da inundação no controle desta espécie exótica. Observamos alta cobertura vegetal no período de seca, porém uma queda na cobertura vegetal de *U. humidicola* posterior a inundação. Para complementarmos este estudo foi elaborado o segundo capítulo, onde foi realizado um experimento em laboratório que simulou a inundação e a influência de macrófitas aquáticas na sobrevivência de mudas de *U. humidicola*. Foi possível verificar que a associação com macrófitas promove a mortalidade de mais de 50% da cobertura aérea de *U. humidicola*, bem como, foi avaliado a persistência do banco de sementes de *U. humidicola*, que indicou alto potencial de germinação de sementes pós-inundação. No terceiro capítulo foi avaliado o banco de sementes das espécies nativas, as estratégias de germinação e as previsões de espécies que podem ocorrer na vegetação quando ocorre inundação e seca foram avaliados através de experimentos em casa de vegetação, com tratamentos de inundação e pós-inundação do solo. Foi encontrada uma comunidade rica em macrófitas durante a inundação e muitas emergentes anuais e perenes pós-inundação. Já no quarto capítulo foi avaliada a regeneração em campo, a distribuição do banco de plântulas sob influência da inundação foi avaliada levando-se em consideração a formação de detritos orgânicos provenientes de macrófitas aquáticas que morrem no rebaixamento das águas, a influência dos detritos sobre a distribuição de plântulas foi avaliada nos diferentes níveis de

topografia. Observou-se que diferentes concentrações de detritos orgânicos sugerem diferentes estratégias de colonização, variando a distribuição de plântulas entre os níveis. E por fim, a importância do banco de sementes na manutenção da comunidade nativa foi analisada no último capítulo, onde foi possível verificar que o banco apresenta alta diversidade e pode ser uma importante fonte de manutenção da comunidade de espécies nativas, porém a *U. humidicola* deve ser tratada com cuidado, pois promove diminuição do banco de macrófitas em campo cultivado, sendo que este grupo de espécies é particularmente importante no controle de colonização de *U. humidicola*. Os resultados encontrados neste estudo podem auxiliar em planos de conservação e preservação de áreas úmidas frente à invasão de espécies exóticas.

## Abstract

Exotic species such as *Urochloa humidicola* (brachiaria) are introduced into native grassland and threaten the diversity of wetlands, such as the Pantanal. In wetlands subjected to extreme seasonal events, seed reserves in the soil are an important source of vegetation regeneration. Information on the influence of exotic plants and flooding in relation to seed bank dynamics, distribution and composition of the field vegetation helps to elucidate the adaptive characteristics aimed at germination, growth and establishment of the species. The aim was to evaluate the primary sources of regeneration: the seed bank and seedlings, under the influence of different levels of topography and seasonality and, establishment of exotic plants. The collections occurred in two grasslands: one dominated by native species and another dominated by *U. humidicola*, in the Pantanal of Mato Grosso do Sul. Sampling occurred in seasonal ponds where three topographic levels were established (low, mid and high); in addition, to analyze the effect of seasonality, the samples were collected at the end of the dry and flood seasons, in two years. Vegetation, seedling bank and seed data were analyzed separately, where it was possible to evaluate each stage of life due to the seasonality, topography and influence of exotic plants. Then the, vegetation and seed bank data were related to verify the importance of the seed bank in the maintenance of the native flora. Thus, the first chapter addressed the influence of *U. humidicola* on native grassland and the role of flooding in the control of this exotic species. We observed high vegetation cover in the dry season, but a decrease in *U. humidicola* vegetation cover after flooding. To complement this study the second chapter was elaborated, where an experiment was carried out in a laboratory that simulated the flood and the influence of aquatic macrophytes on the survival of *U. humidicola* seedlings. It was possible to verify that the association with macrophytes promotes the mortality of more than 50% of *U. humidicola* aerial cover, as well as, the persistence of the *U. humidicola* seed bank, which indicated a high germination potential of post-flood. In the third chapter the seed bank of the native species was evaluated, the germination strategies and the predictions of species that can occur in the vegetation when there is flood and dry were evaluated through greenhouse experiments, with flood and post-flood treatments from soil. A community rich in macrophytes was found during the flood and many emergent annual and post-flood perennials. In the fourth chapter we evaluated the regeneration in the field, the distribution of the seedlings bank under the influence of the flood was evaluated taking into consideration the formation of organic debris from aquatic macrophytes that die in the lowering of the waters, the influence of the debris on the distribution of seedlings was evaluated at different levels of topography. It was observed that different concentrations of organic debris suggest different colonization strategies, varying the distribution of seedlings between the levels. Finally, the importance of the seed bank in the maintenance of the native

community was analyzed in the last chapter, where it was possible to verify that the bank presents high diversity and can be an important source of maintenance of the community of native species, but, *U. humidicola* should be treated with caution, since it promotes a decrease of the macrophytic bank in the cultivated field, and this group of species is particularly important in the control of *U. humidicola* colonization. The results found in this study can aid in the conservation and preservation of wetlands against the invasion of exotic species.

## Introdução Geral

Em zonas úmidas tropicais as inundações anuais são a principal fonte de modelagem da comunidade vegetal (Junk 1996). No Pantanal, a inundação é um evento previsível que promove alterações na estrutura e composição florística (Pott & Silva 2015), em virtude da intensidade e duração do hidropérido (Schessl 1999), da variação topográfica (Pagotto et al. 2011, Bao et al. 2014), do relevo e dos diferentes tipos de solo (Pott & Silva 2015). Em resposta, estas comunidades são estruturadas de acordo com a capacidade de resistência e resiliência (Mouillot et al. 2011), selecionando espécies terrestres (Scarano 1998), anfíbias e aquáticas (Pott & Pott 2004). A compreensão da relação entre a diversidade e a manutenção das comunidades vegetais requer o conhecimento de como as espécies interagem entre si e como estas respondem aos eventos sazonais (Ives & Carpenter 2007). Isto irá depender da história evolutiva das espécies, que podem ficar restritas a áreas específicas e/ou variarem de acordo com determinado padrão de inundação (Wittmann & Junk 2003).

O crescimento e o estabelecimento da vegetação em áreas úmidas podem ser determinados pelo banco de sementes no solo (Harper 1977, Leck & Simpson 1987), que é composto por sementes viáveis (Leck & Simpson 1987), que podem apresentar dormência ou não (Baskin & Baskin 2014), pela capacidade de estabelecimento das plântulas (Muller-Landau et al. 2002), por rebrota ou propagação vegetativa (Middleton 1999), estes mecanismos de regeneração irão determinar as taxas de crescimento das populações (Westcott et al. 1996). Neste sentido, o recrutamento em ambientes sazonais, ao longo do tempo promovem estratégias adaptativas individuais na germinação e colonização de cada espécie (Baskin & Baskin 2014).

A manutenção da diversidade através da dinâmica do banco de sementes, de plântulas e da comunidade vegetal é um fator determinante no reestabelecimento das comunidades (van der Valk & Davis 1978; Leck & Simpson 1987; Middleton 1999). O desempenho do banco de sementes depende da heterogeneidade ambiental que pode influenciar a riqueza de espécies e o padrão espacial que é visto na vegetação (Elsley-Quirk & Leck 2015). No modelo de sucessão de

espécies em áreas úmidas, van der Valk (1978), propôs que o funcionamento do ambiente ocorre como um filtro ambiental variável que se comporta entre dois estados: seco (sem água) e inundado (com água parada), onde as espécies com características adequadas podem se estabelecer e as que não as possuem são excluídas por um período, visto que o acúmulo de sementes no tempo e espaço está relacionado à história de vida da vegetação estabelecida (Leck & Simpson 1987).

A inundação como força seletiva exerce pressão para que algumas espécies colonizem determinadas regiões (Rahel 2000; Myers & Harms 2009), este processo se reflete na diversidade local e, neste caso, as metacomunidades vegetais podem se tornar mais vulneráveis à invasão de espécies com características dominantes e estas espécies passam a exercer uma força de controle na função da zona úmida (Junk et al. 1989; Cronk & Fennessy 2001). Assim, a riqueza e abundância de espécies dos campos úmidos do Pantanal pode ser limitada pela presença de plantas exóticas dominantes que são utilizadas para pastagem (Bao et al. 2015), visto que nos campos nativos é encontrada uma diversa comunidade de plantas herbáceas as quais coexistem com as espécies exóticas (Allem & Valls 1987). Para intensificar a atividade da pecuária estas comunidades passaram a ser substituídas por gramíneas exóticas, principalmente as do gênero *Urochloa* (Pott & Pott 2004), consideradas neste processo uma ameaça à diversidade dos campos nativos do Pantanal, pela rápida colonização e tolerância ao alagamento.

O estudo do banco de sementes pode revelar o potencial de resiliência das plantas nativas e o poder desta espécie exótica em formar um banco de sementes persistente ao longo dos anos capaz de competir com as plantas nativas. Além disso, o banco de sementes pode fornecer informações sobre as mudanças na composição florística durante e após o evento extremo sazonal (Middleton 2003; Hopfensperger 2007). A alta rotatividade de espécies em pastagens de zonas úmidas nos dão informações sobre associação entre as comunidades vegetais e a dinâmica de inundação em um curto espaço de tempo (Lourival et al. 2011), o que torna estes ambientes ideias para estudos ecológicos que visem investigar as estratégias de germinação e

colonização dos campos úmidos e como ocorre a sucessão de espécies durante os períodos de inundação e seca. Diante da importância de contribuir para o conhecimento acerca da flora dos campos inundáveis do Pantanal, tivemos como objetivo avaliar as fontes primárias de regeneração: o banco de sementes e de plântulas, sob influência de diferentes níveis de topografia e sazonalidade e, estabelecimento de plantas exóticas. Para compreendermos a influência destes fatores sobre o estágio de vida das espécies na comunidade, foram elaborados cinco capítulos estruturados da seguinte forma:

(i) O primeiro capítulo, “**Effects of *Urochloa humidicola* on plant diversity in native grasslands in a Neotropical wetland**”, avalia o efeito da introdução da exótica *U. humidicola* sobre a vegetação de espécies nativas, além de analisar a influência da variação sazonal no controle de espécies exóticas e na distribuição da vegetação. Neste capítulo prevemos que a introdução da *U. humidicola* é limitada por inundações e pelo aumento da colonização de espécies nativas adaptadas, contudo, a alta tolerância a inundação desta espécie, nos levou a elaboração de um experimento que complementasse nossos resultados de campo, este experimento foi abordado no segundo capítulo.

(ii) O segundo capítulo: “**Does competition with aquatic macrophytes associated with seasonal flooding limit the invasion potential of *Urochloa humidicola* in the Pantanal grassland?**”, visa entender as estratégias de colonização da exótica *U. humidicola*, para isso um experimento foi conduzido em casa de vegetação para avaliar a persistência do banco de sementes, bem como, o período ideal de germinação. Para verificar se as causas da redução de cobertura vegetal de espécies exóticas em campo esta associada à inundação ou a presença de espécies nativas adaptadas ao período, também realizamos testes de sobrevivência simulando a inundação de mudas de *U. humidicola* na ausência e na presença de macrófitas aquáticas.

(iii) O terceiro capítulo, “**Seed bank of seasonally flooded grassland: experimental simulation of flood and post-flood**”, analisa o banco de sementes de espécies nativas. Este capítulo visa entender a regeneração e sucessão da vegetação nativa a partir do banco, durante e

após o evento extremo sazonal. Um experimento para simular o efeito de inundação e pós-inundação no banco de sementes foi conduzido para verificar quais as mudanças que podem ocorrer na composição da comunidade vegetal. A partir das estratégias de história de vida na comunidade, de acordo com a variação sazonal - ciclo de espécies anuais e perenes e, contrastando a ocorrência de espécies aquáticas, anfíbias e terrestres.

(iv) O quarto capítulo: “**Seasonal flooding, topography and organic debris deposition interact to influence seedling emergence and distribution in a seasonally flooded grassland**”, aborda a regeneração em campo, prevê-se que os eventos extremos sazonais tenham um efeito de longo alcance sobre a distribuição do banco de plântulas, mas isso irá depender das habilidades de regeneração em resposta aos efeitos indiretos da inundação, como através do acúmulo de detritos orgânicos em função da topografia local. Desta forma, foi conduzido um estudo para determinar se as diferentes intensidades de cobertura de detritos orgânicos variantes de acordo com a topografia influenciam a distribuição de plântulas em campo de pastagem.

(v) O quinto capítulo: “**Maintenance of the plant community in wetlands: efficient role of the seed bank in regeneration of native plants**”, avalia a vegetação e o banco de sementes em conjunto, para analisar se a presença da espécie dominante *U. humidicola* na vegetação desempenha um papel importante na criação de diferenças entre a flora acima e abaixo do solo e como o banco pode atuar na manutenção da diversidade de espécies da comunidade nativa, assim hipotetizamos que a dinâmica de inundação e seca promove a entrada de distintas espécies no banco de sementes com grupos alternativos de plantas aquáticas, anfíbias e terrestres que aumentam a riqueza e abundância do banco e conseqüentemente na vegetação, atuando na manutenção da diversidade desta zona úmida.

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## Capítulo I

# **Effects of *Urochloa humidicola* on plant diversity in native grasslands in a Neotropical wetland**

Francielli Bao

Marco Antonio de Assis, Rafael Arruda, Arnildo Pott

### Abstract

Studies on species invasion in wetlands are fundamental to understanding the consequences of the introduction of exotic plants, which are often utilized in grasslands. The loss of native plant diversity is of increasing concern. We evaluated the effect of *Urochloa humidicola* on floristic similarity between native and cultivated grasslands and the differences in spatial distributions between species. To analyze the effect of seasonality, we collected samples at the end of flood and dry periods. In native grasslands and another area cultivated with *U. humidicola*, samples were collected at three elevations (bottom; intermediate; and top). We identified 90 species and observed a high similarity between the cultivated and native grassland during the flood period; both grasslands exhibited significant differences during the drought period. This difference was due to an increased presence of grasses, mainly *U. humidicola*, in the cultivated grassland. Increased similarity between bottom and intermediate elevations was due to an increased proportion of aquatic macrophytes. In contrast, the top elevation differed due to the contribution of terrestrial species. We conclude that the native and cultivated grasslands exhibit differences in vegetation composition and are primarily structured by seasonal flooding, and small variations in elevation promote zonation within the plant community.

**Keywords:** exotic species; flood; floristic similarity; plant invasion; vegetation dynamics.

## Introduction

The Pantanal wetland is a region strongly ruled by hydrology. The seasonal flood is the major ecological phenomenon in this region; therefore, this land is categorized as a seasonal wetland subject to flood pulses (Junk et al. 1989). Extended flood duration is an adverse condition that cannot be overcome by many terrestrial vascular plants (Scarano 1998). Variation in elevation produces permanent and/or periodically flooded zones (Arieira and Nunes da Cunha 2006), that influence the presence and abundance of species, which are distributed along flood gradients and vary according to their tolerance to flooding and drought (Nunes da Cunha and Junk 2001).

Natural grasslands comprise the largest part of the Pantanal flood plain, which is typically utilized for cattle ranching. Several herbaceous plant communities and numerous forage species coexist (Allem and Valls 1987). The low productivity of native grasslands has caused an increase in cultivated grasses, mainly *Urochloa humidicola* (Rendle) Morrone & Zuloaga (Pott and Pott 2004). *Urochloa humidicola* is flood tolerant because of its numerous adventitious roots that become evident during periods of excessive flooding (Mattos et al. 2005). The introduction of these species is a threat to local biodiversity and can lead to the exclusion of native species (Clavero and García-Berthou 2005). Considering the important role of macrophytes in the conservation of wetland biodiversity and ecosystems, the introduction of *Urochloa* species is of concern given their rapid spread in the Neotropics (Michelan et al. 2010).

Temporary flooding in tropical grasslands is common due to excessive water from rainy periods or poor drainage (Dias-Filho 2005). Plants survive water stress via tolerance mechanisms that induce metabolic and morphological adaptations (Crawford 1993). In general, the damage to plants varies according to the frequency, duration and depth of the flood (Anderson 1974). Floods in the Pantanal are recurrent due to seasonal rains and/or river overflow; however, native species exhibit similar responses to water stress due to recurrent flooding as well as flooding from unpredictable events (Arieira and Nunes da Cunha 2006).

The process of flooding reflects in local plant diversity and, in this case, plant communities can become more susceptible to invasion by exotic species (Sharma and Panu 2014). The combination of environmental factors with some kind of disturbance is patterns which determine richness of native and exotic species in a time-space scale (Mack et al. 2000, Shea and Chesson 2002). Therefore, flooding and dispersal and the seed bank of exotic species can affect the distribution of native plants in large scales (Fenner and Thompson 2005, Lilley and Vellend 2009), whereas initial competition and habitat preference influence small-scale distribution (Davies et al. 2005). Deleterious effects of competition on native plants are enhanced after disturbance, which may potentially lead to changes in ecosystem function (Mack et al. 2000; Pimentel et al. 2000).

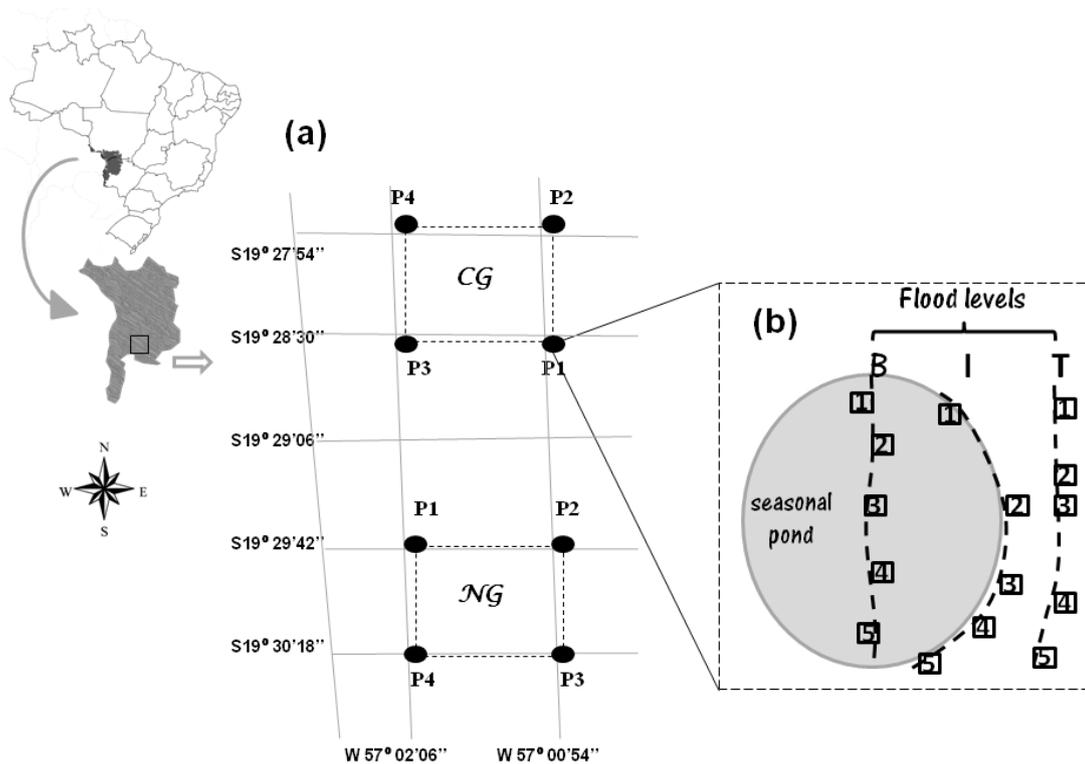
The introduction of an alien aggressive flood-tolerant plant species into wetlands is of great concern because it may become invasive and so interfere with native communities (Zedler and Kercher 2004). Given that flooding has an important role in the Pantanal, and that extensive areas of grassland are being invaded by species of *Urochloa*, it is fundamentally important to understand the impact of invasive grasses in this wetland and how they can affect the native plant community. Therefore, the objective of our study was to evaluate the influence of grasslands cultivated with *U. humidicola* compared to native grasslands after the flood and drought periods in the Pantanal, and to determine the influence of topography on the spatial distribution of species.

We tested the following hypotheses: 1. The introduction of *U. humidicola* is limited by flooding and the increased colonization of adapted native species. 2. The characteristic seasonal variation of the Pantanal results in increased homogeneity in modified grassland during the rainy period. 3. Plant cover trends can be observed at topographic levels, demonstrating that flood depth can limit the establishment of certain species according to the ground level.

## Material and methods

The Brazilian Pantanal wetland exhibits a sub-humid tropical climate with a mean annual temperature of 26°C and a mean annual rainfall of 1100 mm (Allem and Valls 1987). The study area is located in the sub-region of the Abobral in the Pantanal wetland in Mato Grosso do Sul, Brazil. This area is characterized by the presence of grassland areas that undergo a period of flooding (Silva and Abdon 1998). Although the entire plain is flooded, various places are flooded for longer periods than others; these differences in flooding time vary according to elevation. These native grasslands are traditionally utilized as pastures and were partially replaced by cultivated grassland using the exotic *Urochloa humidicola* (Rendle) Morrone & Zuloaga (Poaceae). This species was first introduced 15 years ago.

To analyze the effect of the seasonal flood pulse on the plant species cover in native grassland and cultivated *U. humidicola* areas, we collected samples at the end of a flooding period (late June 2011) and at the end of a drought period (early October 2011). To analyze the influence of exotic *U. humidicola* on the structure of native vegetation under grazing pressure, samples were collected from two grassland areas, 1 Km<sup>2</sup> each, that were utilized for grazing. These grasslands were classified as native grassland and cultivated grassland with *U. humidicola* which are hereafter referred to as NG and CG, respectively. For each grassland area, we chose four seasonally flooded areas (P1, P2, P3 and P4) that were 1 Km apart (Fig. 1a). To evaluate how small differences in elevation affect the distribution of species, we collected samples in each area at the following three ground levels: B, bottom (longer flood duration); I, intermediate; and T, top (shorter flood duration) (Fig. 1b). The group levels were separated by approximately 30 cm vertically and approximately 10 m horizontally.



**Fig. 1** Location of the study area at São Bento ranch in the Abobral sub-region of Pantanal in Mato Grosso do Sul, Brazil; **a** Representative plots in cultivated grassland (CG) and native grassland (NG) with sampling points (P1, P2, P3 and P4); **b** Flood levels (B, bottom; I, intermediate; and T, top) in the eight sampled points.

In the field, during both flooding and dry periods, the plant cover was estimated in a delineated area of 0.50 x 0.50 m. At each flood level, we sampled 5 replicates distributed according to a table of random numbers from 0 to 50 m (Fig. 1b; cf. Goodman et al. 2011). Each quadrat was subdivided into 4 equal quadrants, and data were obtained by visual estimate by the same individual observer.

Specimens from all species were collected and subsequently incorporated in the Herbarium CGMS at the Universidade Federal de Mato Grosso do Sul. The species were identified using specialized literature (*e.g.*, Pott and Pott 1994; Pott and Pott 2000). Individuals were named according to the plant list of Pantanal (Pott and Pott 1999) and Flora of Brazil (Forzza et al. 2010), following the APG III – Angiosperm Phylogeny Group (2009).

The floristic similarities between the two types of grassland (NG and CG) and the two seasons, as well as among the three flood levels were compared. The plant community data were then ordinated by non-metric multidimensional scaling (NMDS) utilizing the Bray-Curtis distance. To determine the number of NMDS solutions utilized as a dependent variable, a linear regression of the original values of the matrix of similarity was performed with the values obtained from the ordination in one, two or three solutions. The best solution was that with the highest  $r^2$  value and the lowest stress value. We performed 4 ordinations (2 for NG and 2 for CG). A simple linear regression was created to test the effects of axes 1 and 2. The species with the highest contributions in similarity were determined using the similarity percentage (SIMPER).

## Results

### Floristic composition

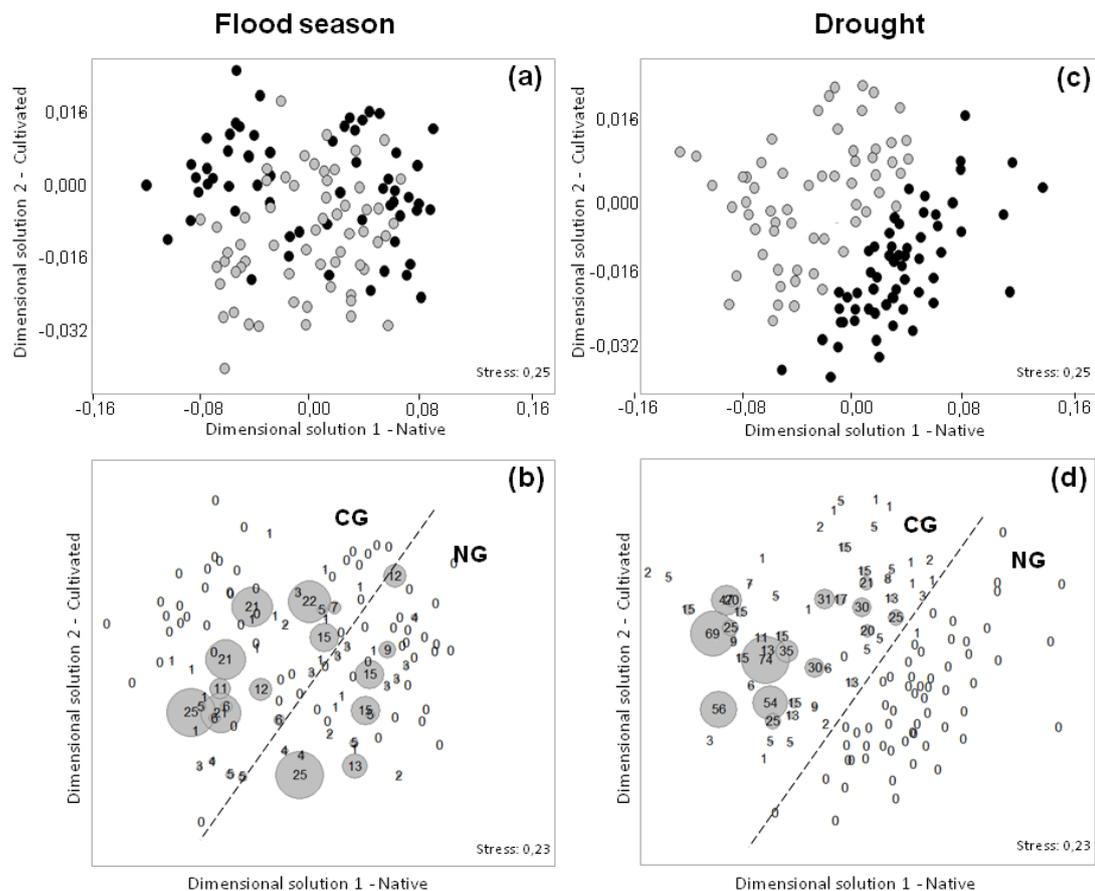
The vegetation was composed of 90 species from 29 families. The CG contained 63 species; 71 species were found in the NG. Only one species was a Pteridophyte fern ally (Isoetaceae). All other species were angiosperms. Families with the greatest species representation included the Poaceae (23 species) followed by the Cyperaceae (9); the Euphorbiaceae and Plantaginaceae families (6 each); the Rubiaceae (5); the Fabaceae, Malvaceae and Onagraceae families (4); and the Alismataceae and Asteraceae families (3). Four other families were represented by 2 species, and 16 families were represented by only one species (Table S1). Approximately 75% of the species identified were perennial, and 25% were annual.

The most frequently observed species included the emergent *Richardia grandiflora*, *Diodia kuntzei* and *Hyptis brevipes*. During the flood period, a greater occurrence of the Cyperaceae family was observed. In terms of frequency, *Helanthium tenellum*, *Echinodorus grandiflorus*, and the aquatic plants *Eleocharis minima* and *E. acutangula* were most common. During the dry period, grass species were more common. The greatest number of species was

observed in the genus *Paspalum*. Increased coverage of *Setaria parviflora*, *Digitaria fuscescens*, *Reimarochloa brasiliensis* and *Urochloa humidicola* was also observed.

#### Relation between cultivated and native grasslands

At the end of the flooding season, there was higher similarity between grasslands. CG achieved 22.3% similarity among species. During the same time period, NG exhibited 20.5% species similarity (Fig. 2a). The similarity between both grasslands was maintained by the aquatic macrophytes *Eleocharis acutangula*, *E. minima*, *Pontederia subovata* and *Echinodorus grandiflorus*. Together, these species contributed to greater than 60% of the similarity observed between NG and CG and were responsible for the observed low percentage (17.8%) of *U. humidicola*. The high percentage of these species in the rainy season resulted in an increased similarity between both grasslands.



**Fig. 2** Ordination by nonmetric multidimensional scaling (NMDS) of the similarity between species (Bray-Curtis index), as calculated from the vegetation cover of each species in relation to

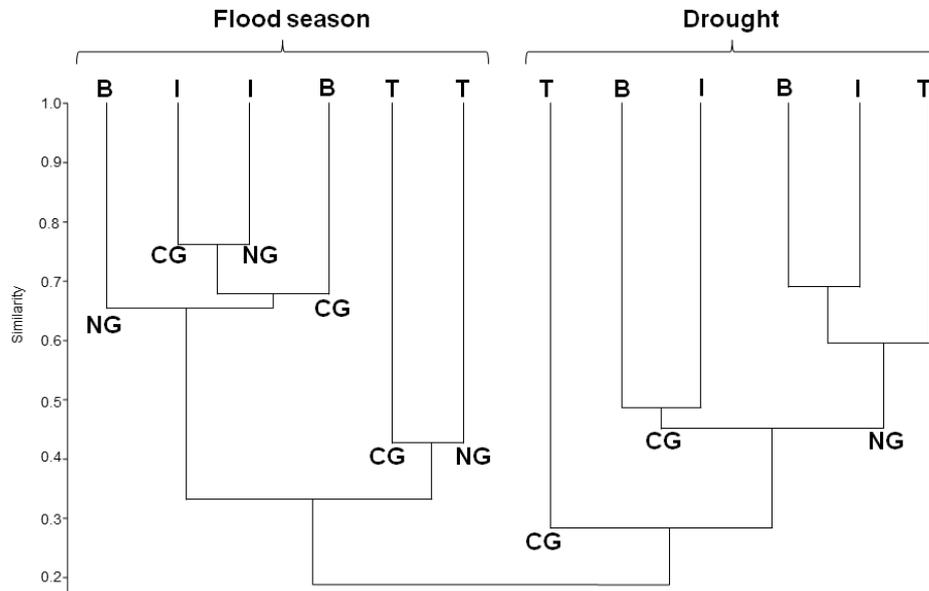
seasonality and the type of grassland; **a** the effect of the flood season on cultivated (●) and native grasslands (○); **b** NMDS diagram illustrating the effect of the flood season on the distribution of *U. humidicola* in cultivated grassland (CG) and native grassland (NG); **c** the effect of the dry season on cultivated (●) and native grasslands (○); **d** the effect of the dry season on the distribution of *U. humidicola* in CG and NG.

The exotic *U. humidicola*, which exhibited minimal cover during flooding, was observed in both CG and NG. These findings demonstrate that the flood in this case resulted in increased homogenization between native and cultivated species (Fig. 2b). At the end of the dry period, the similarity among species in NG was increased (24.7%) compared with CG (18.5%). The reduced similarity in CG was due to the high percentage of *U. humidicola* (Fig. 2c). *U. humidicola* alone contributed to 46.3% of the dissimilarity observed between the grasslands; a significant difference ( $r = 0.44$ ,  $P < 0.001$ ) between NG and CG was observed in the dry period (Fig. 2c). An increase in *U. humidicola* coverage occurred during the dry period due to the limited growth of native species. In NG, we observed increased native species coverage. As native species coverage took over cultivated species, the heterogeneity of the grasslands increased in this period (Fig. 2d).

By comparing the rainy and dry periods, we observed differences in vegetation structure and composition that were independent of the grassland type. During the rainy season, an increase in species similarity (19.6%) was observed; more than 50% of similarity in this period was attributed to *Eleocharis acutangula* (21.2%), *E. minima* (21.0%) and *Pontederia subovata* (19.7%).

During the drought period, species similarity decreased (17.3%). A reduction in the proportion of aquatic macrophytes provided room for terrestrial species such as *Richardia grandiflora* (27.6%), *Reimarochloa brasiliensis* (18.7%) and *U. humidicola* (12.3%), which demonstrated the greatest similarity during this period. These species contributed to the

difference observed between the seasons. Seasonality affects species in a manner that follows a pattern of distribution dependent on flood and drought, thereby forming two groups of distinct species (Fig. 3).



**Fig. 3** Grouping of species based on similarity (Bray-Curtis index) in the two grasslands (cultivated grassland (CG) and native grassland (NG)) within the flood levels (B, bottom; I, intermediate; T, top). Distinct groups were formed by the seasonality effect (flood and drought seasons).

#### Effect of flood levels on the spatial distribution of species

The greatest similarity among species was observed at the I elevation (16.3%); the lowest similarities were observed at the T (16.1%) and B levels (14.5%). The emergent *R. grandiflora* demonstrated the greatest contribution towards similarity (34.4%), with increased representation at the T level; contributions to B and I were high for *P. subovata* (17.9%) and *E. acutangula* (16.9%), respectively. Furthermore, the stoloniferous Rubiaceae *R. grandiflora* and *Diodia kuntzei* were the only species identified at all levels in both grasslands and during both seasons.

Some perennial species were exclusive to a given elevation. For example, *Hyptis lorentziana*, *Discolobium pulchellum*, *Nymphaea gardneriana* and *Ludwigia nervosa* were

exclusively observed on the B level. The legume *Mimosa pigra* was exclusively observed on the I level. The sedges *Cyperus cornelii-ostenii* and *Schoenoplectiella supina* and the fern ally *Isoetes pedersenii* were exclusively identified on the T level (Table S1).

The largest difference in species community composition observed between elevation treatment levels occurred between T and B (91.7%); the species that provided the greatest contributions to this difference included *R. grandiflora* (8.9%), *P. subovata* (6.2%), *R. brasiliensis* (6.2%), *E. minima* (5.5%) and *U. humidicola* (5.5%). The smallest difference in species similarity occurred between relief levels B and I (85.2%), where *R. brasiliensis* (11.4%), *E. acutangula* (10%), *P. subovata* (9.3%) and *U. humidicola* (6.2%) contributed to 50% of the difference. By grouping species, we found a high correlation between the B and I levels that is independent of the grassland type and the occurrence of flooding. Given that T does not resemble the other levels, a separate group is formed (Fig. 3).

## Discussion

### Floristic composition

Our study describes differences in the floristic diversity and the structural characteristics of the vegetation noted between CG and NG; these differences are dictated by the flood pulse. All of the species listed in this text are present on the list of Pantanal plants (Pott and Pott 1999) and plants of the Brazilian flora (Forzza et al. 2010).

The occurrence of grasses and sedges is common in seasonally flooded open habitats such as grasslands (Ladeua and Ellison 1999). The observed species composition is in agreement with previous studies elsewhere in the region (e.g. Pagotto et al. 2011). Grasses and legumes represent the greatest number of species in the Pantanal (Pott & Pott 1999), e.g. *Paspalum* spp. *Axonopus purpusii*, *Reimarochla acuta* and *R. brasiliensis* (Valls 1994). Legumes are frequently present under certain types of disturbances, such as flooding and grazing (Allem and Valls 1987). Additionally, the high cover of *Richardia grandiflora*, *Cynodon dactylon* and *Hyptis*

*brevipes* reflects excessive grazing and increased trampling and harrowing (Pott and Pott 1994). *Isoetes pedersenii* was the single Pteridophyte species sampled in this field; plants in this genus commonly appear in seasonally flooded areas (Choi et al. 2008).

#### Relationships between cultivated and native grasslands

Many emergent exotic species are capable of outcompeting native vegetation due to fast growth (Cronk and Fennessy 2001). We observed homogenization and heterogenization between NG and CG that could be explained by seasonal variation. Although flooding reduces the *U. humidicola* coverage in CG compared to the dry period, this plant soon resumes its dominance due to its adventitious roots (Mattos et al. 2005) that suppress native species (Bennett et al. 2012).

Although *U. humidicola* presented in some patches during flooding in NG, it did not succeed during the dry period. Such plants must pass through filters of adaptability, meeting the necessary conditions to germinate and compete with native plants for success and colonization (Lambers et al. 1998). In this case, native species exhibit greater adaptability to seasonal variation. Furthermore, the low cover of *U. humidicola* in the NG may be an example of increased local diversity inhibiting exotic invasion (MacArthur and Wilson 1967; D'Antonio and Vitousek 1992). The seasonal flood, which forms temporary ponds and creates a difficult habitat for the adaptation of exotic species (Junk et al. 2006), is another factor that may inhibit exotic invasion in the NG.

Increased species richness was observed in NG compared with CG; this observation was likely due to the increased coverage of *U. humidicola* in CG, which may limit the germination of other species. Furthermore, *U. humidicola* is allelopathic (Souza Filho et al. 2005). A single dominant invasive species can exert competitive pressure, reducing spatial heterogeneity (McGranaham et al. 2012).

The differences observed in vegetation structure between flooding and dry periods are due to the similarity of some species. Even though the flooding period only lasted 2 weeks on the T level and 2 months on the B level, the structure and composition of the vegetation was modified. The aquatic plants that were abundant during flooding, such as *Bacopa*, *Cyperus*, *Echinodorus*, *Eleocharis*, *Ludwigia* and *Nymphaea* species, were replaced by numerous pioneer and emergent grasses during the dry period. In secondary succession, which encompasses the replacement of species after any disturbance affecting the existing vegetation, the composition and density of species determine the plant community structure (Gleason 1926).

#### Effect of flood levels on the spatial distribution of species

As expected, the different types of hydrological regimes and topographies reveal a plant community with high selectivity within flood levels. With regard to grasslands, either flood or drought periods offer harsh conditions for plant establishment. The topographic variation in areas under seasonal floods promotes a pattern of species zonation in the structure of plant communities (Cattanio et al. 2002). The response to post-flood events, such as drought, is also equally important in seasonal habitats (Bunker and Carson 2005). Due to undeveloped root systems, drought conditions can be more stressful and deadly to young plants than older plants. Given this disparity, intolerant species can be excluded from the community (Lieberman and Li 1992).

We observed a lower number of species in the B level during the rainy period. This observation was likely due to an excess of dead matter that was deposited as the water receded. However, species richness at the B level was increased in the dry period. This occurs in areas flooded for longer periods, as such areas receive a greater number of carried seeds (Pagotto et al. 2011). Seed build-up varies according to income (through dispersal) and output (Luken 1990). Both situations vary widely year round, and succession is likely regulated by seasonal patterns of

seed income (Young et al. 1987). Such patterns result in variations in abundance, species richness and vegetation composition (Pagotto et al. 2011).

The high similarity observed between the B and I levels is common in periodically flooded areas. This similarity is attributed to the emergent plants *Hyptis brevipes*, *Reimarochloa brasiliensis* and *Richardia grandiflora* and the aquatic amphibious species *Diodia kuntzei*, *Echinodorus grandiflorus*, *Eleocharis acutangula*, *E. minima*, *Ludwigia octovalvis* and *Melochia simplex* (Pott and Pott 1994; Pott and Pott 2000). Sedges are frequently observed in grasslands after a flood period (Crispim et al. 2002). The diversity of aquatic plants associated with flood pulses favor the development of macrophytes with various life forms (Junk et al. 1989).

Differences observed in the T level were attributed to the diversity of grass species identified in the grassland. Such diversity is characteristic of a dry habitat with little or no flooding (Allem & Valls 1987, Crispim et al. 2002, Gasparino et al. 2006, Schessl 1999).

A lack of oxygen and an increase in the concentration of phytotoxic components in flooded soils hinder both root growth and the physiological functions of plants (Mendelssohn et al. 1981). These conditions select for various types of plant metabolism (Joly 1994). Consequently, we hypothesize that small variations in topography exert large effects on plant community structure under flood conditions. A striking characteristic in wetlands is the distribution of plants in well-defined zones (Spence 1982). Alterations in water levels can create conditions that reduce or eliminate the possibility of seed production, germination and establishment (Barret et al. 1993).

The seasonal effects function as a filter at different topographic levels, favoring the establishment and development of native species due to enhanced adaptation. However, *U. humidicola* relies on vegetative propagation, which is more efficient than seed germination. Because CG presents with a history of planting, this exotic grass becomes dominant. The richness of exotic species is considerably increased in all spatial scales in grasslands with an overgrazing history (Altesor et al. 2006), whereas the richness of native species is reduced.

Nevertheless, flood delays the establishment of *U. humidicola*. If the propagation mechanism of this plant eventually fails, native diversity is restored. The abundance of seeds and seedlings suggests that the seed bank plays an efficient role in the regeneration of diversity (Bao et al. 2014).

Although diversity is temporarily lost in natural grasslands that are subject to disturbance via the introduction of an exotic species and/or flooding, these areas are recolonized with a high proportion of emergent species and native grasses. These native plants regenerate quickly, mainly due to local richness and abundance. In accordance with our hypotheses, as well as the seasonal cycle, the variation between the characteristic flooding and dry periods in the Pantanal wetland acts as a determining factor to increase the similarity between native and cultivated grasslands. The variation in topography reveals a community structured within each level, which varies with flooding.

### **Agknowledgements**

The authors thank to INAU (Instituto Nacional de Ciência e Tecnologia de Áreas Úmidas, or Brazilian Institute for Wetlands), for support and finance of the project, and to the Brazilian governmental agencies CNPq for the scholarship (F.B.) and research grants (A.P. and M.A.A.) and CAPES for Visiting Professor grant (A.P.).

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## **Supplementary Material**

**Table S1** Species present in native and cultivated grasslands based on flood gradients (B, bottom; I, intermediate; T, top) under seasonal variation (flooding and drought seasons) in the Abobral sub-region of the Pantanal wetland in Mato Grosso do Sul, Brazil.

FAMILY/SPECIES	Life Cycle	Flooding						Drought					
		Native			Cultivated			Native			Cultivated		
		B	I	T	B	I	T	B	I	T	B	I	T
<b>ACANTHACEAE</b>													
<i>Justicia laevilinguis</i> (Nees) Lindau	perennial								X				
<b>ALISMATACEAE</b>													
<i>Helanthium tenellum</i> (Martins) Britton	perennial	X	X	X	X		X						
<i>Echinodorus glaucus</i> Rataj	perennial		X										
<i>E. grandiflorus</i> (Cham. & Schltdl.) Micheli	perennial	X	X	X	X	X	X	X	X		X	X	
<b>AMARANTHACEAE</b>													
<i>Pfaffia glomerata</i> (Spreng.) Pedersen	perennial										X		
<b>APIACEAE</b>													
<i>Eryngium ciliatum</i> Cham. & Schltdl.	perennial			X						X			
<b>APOCYNACEAE</b>													
<i>Metastelma berterianum</i> (Spreng.) Decne.	annual							X	X				
<b>ASTERACEAE</b>													
<i>Praxelis clematidea</i> R.M. King & H. Rob.	annual								X	X		X	
<i>Sphagneticola brachycarpa</i> (Baker) Pruski	perennial		X			X	X	X	X	X	X	X	
<i>Stilpnopappus pantanalensis</i> H. Rob.	perennial							X		X		X	
<b>COMMELINACEAE</b>													
<i>Commelina erecta</i> L.	perennial		X	X									
<b>CONVOLVULACEAE</b>													
<i>Aniseia martinicensis</i> (Jacq.) Choisy	perennial										X	X	
<i>Ipomoea subrevoluta</i> Choisy	perennial										X	X	
<b>CYPERACEAE</b>													
<i>Cyperus cornelii-ostenii</i> Kük.	perennial			X			X						
<i>C. haspan</i> L.	Perennial	X	X		X	X	X						
<i>C. surinamensi</i> Rottb.	perennial		X	X			X		X	X	X	X	
<i>Eleocharis acutangula</i> (Roxb.) Schult.	perennial	X	X		X	X	X	X	X		X	X	



<i>M. simplex</i> A. St.-Hil.	perennial		X			X	X	X	X	X	X	X	X
<i>M. villosa</i> (Mill.) Fawc. & Rendle	perennial											X	X
<i>Sida rhombifolia</i> L.	perennial										X	X	X
<b>NYMPHAEACEAE</b>													
<i>Nymphaea gardneriana</i> Planch.	perennial					X							
<b>ONAGRACEAE</b>													
<i>Ludwigia grandiflora</i> (Michx.) Greuter & Burdet	perennial					X	X				X	X	
<i>L. nervosa</i> (Poir.) H. Hara	perennial										X		
<i>L. octovalvis</i> (Jacq.) P.H. Raven	annual	X	X	X	X	X	X	X					
<i>L. peploides</i> (Kunth) P.H. Raven	perennial	X	X										
<b>OROBANACEAE</b>													
<i>Agalinis glandulosa</i> (G.M.Barroso) V.C. Souza	annual								X	X			X
<b>PHYLLANTHACEAE</b>													
<i>Phyllanthus stipulatus</i> (Raf.) G.L. Webster	annual				X				X	X			
<b>PLANTAGINACEAE</b>													
<i>Angelonia salicariifolia</i> Bonpl.	perennial				X			X	X	X	X		X
<i>Bacopa australis</i> V.C. Souza	perennial	X	X				X	X					
<i>B. myriophylloides</i> Wettst.	perennial					X							
<i>B. salzmannii</i> (Benth.) Wettst. ex Edwall	perennial				X								
<i>B. stricta</i> (Schrad.) Edwall	annual	X									X		
<i>Scoparia montevidensis</i> (Spreng.) R.E. Fr.	annual								X	X			X
<b>POACEAE</b>													
<i>Andropogon hypogynus</i> Hack.	perennial										X		
<i>Axonopus leptostachyus</i> (Flüggé) Hitchc.	perennial			X				X		X			
<i>A. purpusii</i> (Mez) Chase	perennial			X		X			X	X		X	X
<i>Cynodon dactylon</i> (L.) Pers.	perennial			X					X		X	X	
<i>Digitaria bicornis</i> (Lam.) Roem. & Schult.	annual												X
<i>D. fuscescens</i> (J. Presl) Henrard	perennial							X	X	X	X		
<i>D. horizontalis</i> Willd.	perennial									X			
<i>Echinochloa polystachya</i> (Kunth) Hitchc.	perennial							X	X		X		
<i>Eragrostis articulata</i> (Schrank) Nees	annual									X			



<i>Staelia thymoides</i> Cham. & Schltdl.	perennial	X	X	X	X	X		X	X
<b>VERBENACEAE</b>									
<i>Lippia alba</i> (Mill.) N.E. Br. ex Britton & P. Wilson	perennial			X			X	X	

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## Capítulo II

**Does competition with aquatic macrophytes associated with seasonal flooding limit the invasion potential of *Urochloa humidicola* in the Pantanal grassland?**

Francielli Bao

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Pott

### Abstract

In the Pantanal, agriculture and livestock have contributed to the introduction and spread of exotic grasses. The growth and persistence of these invasive species may depend on their ability to tolerate large seasonal variations in floods. *Urochloa humidicola* is an exotic grass that has been observed to decline in density after seasonal flooding. We aim to determine if *U. humidicola* forms a seed bank that persists to extreme seasonal events and how the presence of aquatic macrophytes affects the survival of *U. humidicola* under flooded conditions. The soil seed bank of a pasture with introduced *U. humidicola* was sampled at the end of the flood and the dry seasons for two years. The emergence of the seed was evaluated for twelve months in greenhouse. The *U. humidicola* cuttings were submitted to floods with and without the presence of aquatic macrophytes for three months. The seedling density of *U. humidicola* was higher post-flood (245 seedlings/m<sup>2</sup>) than the post-dry season (130 seedlings/m<sup>2</sup>). Germination occurred throughout the year, with highest average in 5<sup>th</sup> (62 seedlings/m<sup>2</sup>), 6<sup>th</sup> (104 seedlings/m<sup>2</sup>) and 7<sup>th</sup> months (48 seedlings/m<sup>2</sup>). The presence of aquatic macrophytes under flooded conditions significantly increased mortality of *U. humidicola* (ca. 70%) seedlings compared to isolated floods. These findings suggest that the germination and survival potential of *U. humidicola* under flooded conditions is high, but may be limited by the presence of aquatic macrophytes. A control plan and management of this exotic species in natural pastures of the Pantanal will depend on the natural hydrological and biological controls.

**Keywords:** aquatic macrophytes; flood tolerance; grassland; seed bank; wetland

## Introduction

Invasive plants cause changes in the structure and composition of vegetation due to their dominant characteristics of rapid colonization and propagation (Olden and Poff 2003) causing loss in local plant diversity, by inhibiting the germination and development of native plants (Hejda and Pysek 2006). In wetlands, plant species invasion can be high with habitats hydrologically connected and water functioning as corridors for seed dispersal occasionally over long distances (Brock 2011; Capon and Brock 2006; Middleton 2003). In order to make decisions regarding the restoration and conservation of the native flora of wetlands, it is necessary to know the effect of invasive species in an individual way and their characteristics to colonize a particular community (Hejda and Pysek 2006; Hejda et al. 2009).

The African grass *Urochloa humidicola* was introduced in the Pantanal pastures of the Abobral sub-region in the last 15 years due to increased cattle grazing and high flood tolerance (Bao et al. 2015; Dias-Filho and Carvalho 2000). In the Pantanal the grass has a negative effect on the native plant community in periods of drought (Bao et al. 2015), due to rapid vegetative propagation after flooding (Costa et al. 2011). Is a perennial plant with a low percentage of germination (11%) without scarification treatment (Macedo et al., 2011). However, the species increased their germination by 60% when stored for more than 21 months, as observed in laboratory tests (Costa et al., 2011).

In the field it has already been observed that *U. humidicola* forms a bud bank that continues grow until flooding, despite its high tolerance to floods, the percentage coverage of *U. humidicola* is reduced by up to five times at the end of the flood season (Bao et al. 2015). The cause of its reduced cover during flooding is unknown. One hypothesis is that there is a reduced seed bank after continuous submersion during the flood season. It is not known if *U. humidicola* forms a persistent seed bank and, further, if seed germination declines after submersion. After the flood, *U. humidicola* had increased growth (76%) (Valle et al. 2015) and the possible formation of a persistent seed bank may give it greater success in colonization. For managing

species diversity in seasonally flooded wetlands, it is critical to understand changes in the seed bank dynamics with seasonal changes in hydrology, particularly because seed banks is a life history characteristic common to most species present in the grasslands (Brock 2011).

In grasslands when a dominant species achieves its optimal dormancy strategy within a changing environment, it tends to survive and reproduce more in some years and less in others, but this strategy will depend on how the second dominant competitive species respond (Venable 1989). Applying this concept to our study, where *U. humidicola* is the main dominant species, the variable environment is the flood and dry state of the Pantanal and our dominant secondary species encompasses the aquatic macrophytes, we predict that the existence of *U. humidicola* can be limited by aquatic macrophytes, which present high resilience during flooding (Bao et al. 2014; Oliveira et al. 2015; Souza et al. 2016). That is because they exhibit rapid dispersion and growth due to some specific adaptations (Pieterse and Murphy 1990). Their diversity becomes fundamental in the maintenance of ecological processes, especially when involving the entry of exotic species (Urban et al. 2006).

Thus, we can answer our guiding question of how these Pantanal pasture fields persist over years of introduction of exotic species in places where flood conditions may favor the dispersal of seeds of invasive species. For this study, we conducted a field survey to examine whether the *U. humidicola* seed bank persists to extreme seasonal events. In the greenhouse, the emergence of the seed bank was evaluated for a period of twelve months, to evaluate if the bank presents an ideal period strategy to germinate. We also performed survival tests simulating the flooding of *U. humidicola* seedlings with and without the presence of aquatic macrophytes to disentangle the effects of flooding alone and the presence of aquatic macrophytes under flooded conditions, which may have led to a reduction in coverage *U. humidicola* as observed in the field.

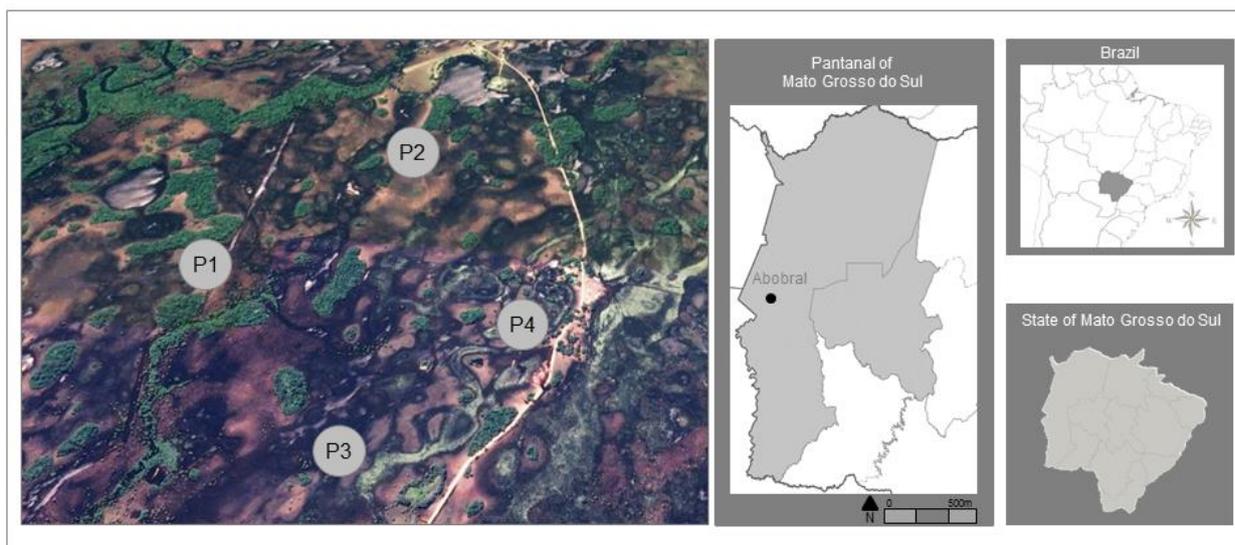
We hypothesize that (i) *U. humidicola* forms a seed bank that persists to extreme seasonal event, we expect greater germination at the end of the flood as a strategy of colonization of the

species; (ii) under experimental conditions, the evidence that occurs the formation of a seed bank capable of germinating throughout the year; and (iii) the presence of aquatic macrophytes reduces the survival of *U. humidicola* under flooded conditions.

## Methods

### Study area

Seed bank samples were collected in a seasonally flooded grassland at ranch Fazenda São Bento (19°29'27.3"S; 57°01'55.9"W), in the Pantanal (Central-West Brazil, Fig. 1). The climate is sub-humid tropical, with an average annual temperature of 26°C (Allem and Valls 1987) and mean annual rainfall of 1100 mm, with maximum (7.34m) and minimum level (2.37m) of the Miranda river (data collected at ranch São Bento, between 2005 and 2015, Fig.S1). The grassland is flooded annually in the summer (between February and May), from both local rain and river overflow (Silva and Abdon 1998). The grassland is characterized by relatively high plant species richness with aquatic, amphibious, and terrestrial plants promoted by a rich soil seed bank, subtle topographic variations, and seasonal ponds at low elevations (Bao et al. 2014). In dry periods, a common characteristic is the rapid spread of grasses (Pott and Silva 2015). Exotic grasses are replacing the natural grassland and *Urochloa humidicola* has been sown in ranch Fazenda São Bento since ca. 15 years ago. The last introduction of *U. humidicola* commercial seeds in the study area was two years before our first sampling.



**Fig 1** Study area with the four seasonal ponds (P1, P2, P3 and P4), distributed in a field cultivated with *U. humidicola* in the Pantanal wetland (Central-West Brazil), on the ranch Fazenda São Bento. On the left, the State Park Road; on the top, the green gallery forest of Abobral river; small dark green spots are forest islets over the brown grasslands. (Image Source: “Fazenda São Bento”, south Pantanal, (19°29′27.3”S; 57°01′55.9”W) Google Earth (accessed 15 March 2017)).

### Sampling

To evaluate the persistence of the seed bank of *U. humidicola*, top soil samples were collected twice at the end of the dry season in October (2013 and 2014) and twice at the end of the flood period in July (2014 and 2015). Four seasonally flooded areas (with ponds in the flood period) with cultivated pasture were sampled (**pond 1** 19°29′23.3”S; 57°01′51.6”W, **pond 2** 19°29′25.3”S; 57°01′19.9”W, **pond 3** 19°28′39.8”S; 57°02′14.2”W and **pond 4** 19°28′06.0”S; 57°02′00.5”W, Fig. 1). The Pantanal because it presents several differences in the topography of the terrain, forms several seasonal ponds throughout the field (Pott and Silva 2015), which present variation in the structure of the vegetation (Bao et al. 2015), in order to achieve greater amplitude variation of seed capture we collected along three contour lines (that is, transects),

representing relative elevation differences of low, mid, and high. In each area (seasonal pond) we collected soil samples randomly.

Transects were marked during the flood season, observing the water mark, in the lowest zone, in the middle of the pond-low, one at the pond edge-mid and one in the higher zone, on the external part-high (e.g. Bao et al. 2014). We collected five soil samples per transect for a total of 15 samples per pond seasonal and 60 samples per seasonal period (flood and dry). A slide of soil was collected inside 0.20 x 0.20m frames, 3 cm deep (e.g. Bao et al. 2014). The samples were kept in plastic bags and transported to the greenhouse.

#### Evaluation of the seed bank

To evaluate the seed bank we used the method of seedling emergence (Thompson et al. 1997) in the greenhouse. The soil samples were spread into plastic trays (30cm x 30cm x 10cm) over a 3 cm layer of sterilized sand, under natural temperature. An automatic sprinkle irrigated the greenhouse twice a day, and the trays were perforated to avoid waterlogging and weekly rotated. The seedlings were counted and removed weekly to avoid competition with new seedlings (Thompson et al. 1997). As the objective was to evaluate the of the *U. humidicola* seed bank, only the grass seedlings were counted and evaluated. The seedlings of other species were removed from the trays to avoid competition with *U. humidicola*. To evaluate the persistence of the seed bank to the extreme seasonal event, the samples were collected at the end of the dry and flood seasons (dry / 2013; flood / 2014; dry / 2014 and flood / 2015). And to evaluate the germination strategy of the seed bank of the species and to verify if it could be delayed, we recorded emergence for twelve months.

#### Evaluation of the tolerance to flooding time and effect of coexistence with aquatic macrophytes

Evaluation of tolerance to flooding time of *U. humidicola* and its coexistence with native aquatic macrophytes was made under two treatments: presence and absence of aquatic

macrophytes. The experiment had three tanks per treatment, with enough water to submerge the cuttings of *U. humidicola* (ca. 0.90m tall). The tanks were under full sun, only covered with mosquito screen. We submerged five trays with five cuttings each, total of 25 cuttings per tank. The grass already had inflorescence. The seedlings removed from the study of the seed bank were used, which were transplanted in trays for this study. To evaluate flood tolerance, the seedlings were kept under total submersion during the experiment. In the tanks with absence of macrophytes we placed five trays with five seedlings each. In the treatment with presence of aquatic macrophytes we placed trays with: *Sagittaria guayanensis*, *Limnocharis flava*, *Hydrocleys parviflora* and *Pontederia subovata*. These species were chosen for being abundant in the Pantanal and for presenting floating leaves, and thereby increased cover and decreased light incidence.

In the three tanks a mean cover of ca. 70% of aquatic macrophytes was standardized. The experiment was carried out for three months, representing the average time the grassland remains flooded (data collected at ranch Fazenda São Bento, 2013-2015). Mortality of individual grasses was considered when the aerial parts were dead (Baruch 1994).

#### Data analysis

To test the temporal variation of germination from the seed bank, two analyzes were performed: (i) seedling density after emergence from the seed bank was tested for twelve months at the end of the dry period and flood, months that presented germination = 0; (ii) seasonal and annual differences in seed bank density (n = 60).

The survival of *U. humidicola* seedlings was estimated in both treatments using the Kaplan-Meier non-parametric estimator (Kaplan and Meier 1958), using the survfit function and the survival curves were compared with log rank test (Harrington 1982), applying the survdiff function of the survival package (Therneau 2015). The log-rank test calculates a value of  $\chi^2$  for

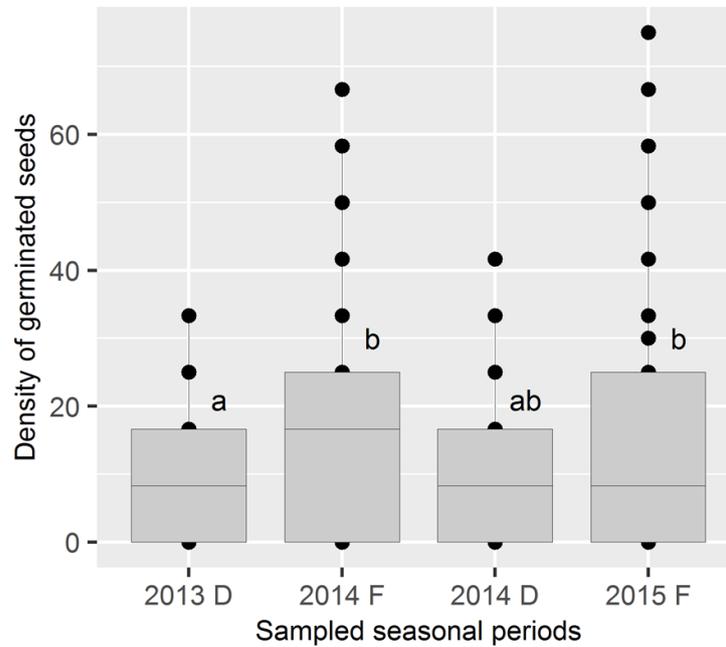
all events in each group. The sum of these values is used to generate a value  $\chi^2$  of comparison between the two curves.

All analyses were conducted in the R environment (R Core Team 2016). For germination analyses of the seed bank, we used generalized linear models (GLMs) with Tweedie distribution (Smyth and Jørgensen 2002), implemented with the `statmod` package (Giner and Smyth 2016). Models with this distribution are indicated for continuous positive data with many zeros (Dunn and Smyth 2005). The results of the comparison models of observation times and seasonal variation were submitted to post-pairing and Bonferroni p-adjustment multiple comparison test using the `lsmeans` package (Lenth 2016).

## Results

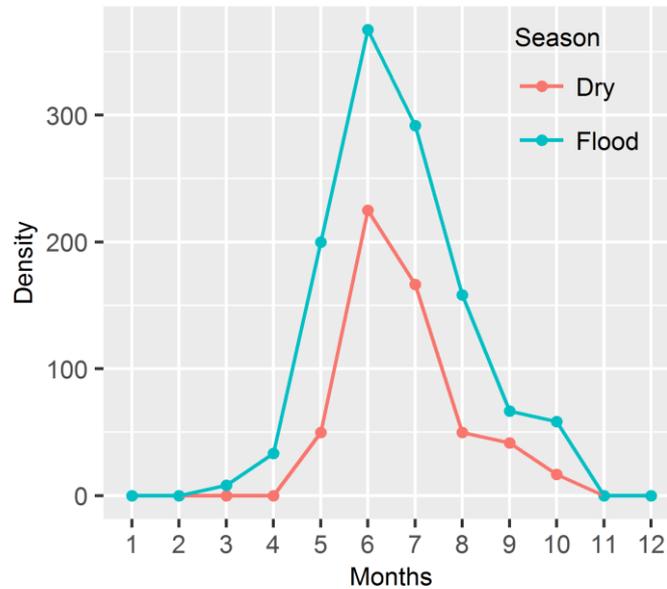
Evaluation of persistence of the seed bank and of germination time

Seeds of *U. humidicola* germinated from the seed bank in both seasons (post-flood and post-dry) in all years of sampling. The total density of germinated seeds in post-flood season (245 seedlings/m<sup>2</sup>) was nearly twice as high as that in post-dry season (130 seedlings/m<sup>2</sup>). There was no difference between the same periods (dry vs dry; flood vs flood). In periods other than 2013D presented the lowest germinated seed density, thus differing significantly from the post-flood periods: 2013D vs 2014F (z-ratio = 3, 55, p = 0.002, Fig. 2) and 2013D vs 2015F (z-ratio = 3.51, p = 0.002, Fig. 2), being greater in the 2014F and 2015F times around 2013D.



**Fig 2** Mean density of germinated *Urochloa humidicola* seeds in greenhouse between the different post-dry (D) and post-flood (F) seasons of the seed bank sampled in grassland in the Pantanal wetland. The different letters reflect the significant difference between the groups.

The density of *U. humidicola* seedlings increased over the months of the greenhouse experiment. It can be observed that in the 3<sup>th</sup> month when germination began in both seasonal periods the density was 8 seedlings/m<sup>2</sup> (post-flood) and 5 seedlings/m<sup>2</sup> (post-dry). In the 6<sup>th</sup> month, the largest period of seed germination (364 seedlings/m<sup>2</sup>) post-flood and (226 seedlings/m<sup>2</sup>) post-dry occurred, and thereafter was a decline in germination, when we observed that the seed bank is depleted in the 11<sup>th</sup> month (Fig. 3).



**Fig 3** Total density of germinated *Urochloa humidicola* seeds in greenhouse, for 12 months, from soil seed bank collected in the flood and dry seasons in grassland in the Pantanal wetland.

The high total density found in the post-flood in the 5<sup>th</sup> (62 seedlings/m<sup>2</sup>), 6<sup>th</sup> (104 seedlings/m<sup>2</sup>) and 7<sup>th</sup> months (48 seedlings/m<sup>2</sup>) contributed to the significant difference between the seasonal periods (Table 1). If we simulate the mean of the months of flooding and drought in the field versus the months of higher and lower germination of *U. humidicola*, we observed that the germination peak of the species can occur in the flood-dry interval (Fig.S2).

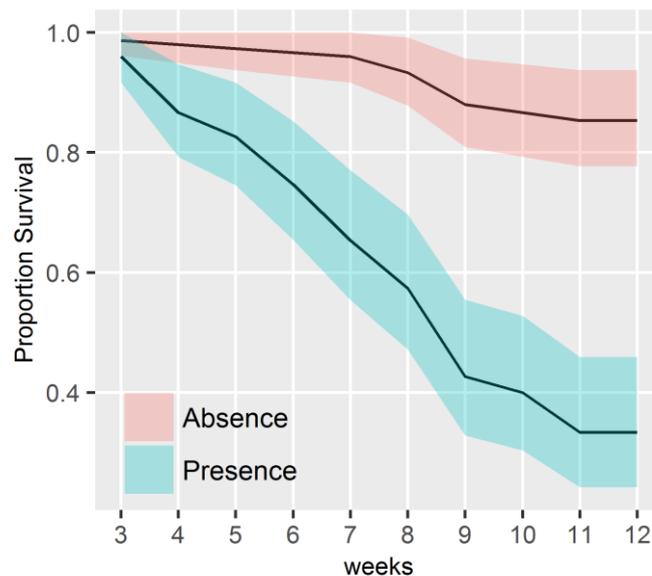
**Table 1** Generalized Linear Model (GLM) between the months for flood and dry seasons.

Season		Estimate	Std. Error	t value	Pr(> t )
Post-flood	10 <sup>th</sup> (intercept)	0.377	0.441	0.8560	0.393
	3 <sup>th</sup>	-1.946	1.146	-1.6990	0.090
	4 <sup>th</sup>	-0.560	0.718	-0.7800	0.436
	5 <sup>th</sup>	1.232	0.508	<b>2.4260</b>	<b>0.016</b>
	6 <sup>th</sup>	1.840	0.481	<b>3.8280</b>	<b>&lt;0.001</b>
	7 <sup>th</sup>	1.610	0.490	<b>3.2880</b>	<b>0.001</b>
	8 <sup>th</sup>	0.999	0.523	1.9110	0.057
	9 <sup>th</sup>	0.134	0.605	0.2210	0.825
	Post-dry	10 <sup>th</sup> (Intercept)	-0.876	0.839	-1.044
5 <sup>th</sup>		1.099	0.983	1.118	0.265
6 <sup>th</sup>		2.603	0.878	<b>2.964</b>	<b>0.003</b>

7 <sup>th</sup>	2.303	0.890	<b>2.587</b>	<b>0.010</b>
8 <sup>th</sup>	1.099	0.983	1.118	0.265
9 <sup>th</sup>	0.916	1.006	0.911	0.363

Evaluation of the tolerance to flooding time and effect of coexistence with aquatic macrophytes

*Urochloa humidicola* showed a high flood tolerance, but the presence of aquatic macrophytes resulted in aerial mortality of ca. 70% of cuttings. The proportion of survivors at week 3 in both treatments was high (0.99 absence) and (0.96 presence). Survival rate declined gradually over time such that by week 8.57% survived in the presence of aquatic macrophytes, while 88% survived in their absence. Based on the difference between the survival curves, mortality was significantly different between treatments ( $X^2 = 44.2$ ;  $p < 0.001$ , Fig. 4), with higher mortality in the treatment with macrophytes.



**Fig 4** Kaplan-Meier survival curves and confidence intervals of *Urochloa humidicola* seedlings under flooding in treatments with presence and absence of aquatic macrophytes from the Pantanal seasonally flooded grassland. Shaded areas represent 95% confidence intervals.

The number of survivors at the end of the experiment was (85% absence and 33% presence) and death (14% absence and 66% presence) (Table 2). This finding indicates high

survivorship of *U. humidicola* after 12 weeks of continuous flooding, and that survivorship is significantly reduced by ~70% cover of aquatic macrophytes. Because macrophytes were planted in separate containers, the pressure of competition in this case was mainly associated with competition for light rather than nutrient resources.

**Table 2** Log-rank test between survival curves of *U. humidicola*.

	N	n.censor	Observed	Expected	(O-E) <sup>2</sup> /E	(O-E) <sup>2</sup> /V
Absence	75	64	11	35.6	17	44.2
Presence	75	25	50	25.4	23.9	44.2

n=total number of seedlings per treatment. Kaplan-Meier estimates of survivor of treatments compared using Log-Rank Mantel-Haenszel test.

## Discussion

Evaluation of persistence of the seed bank and of germination time

Native and exotic species in a flooded field may exhibit different germination patterns over time because of different responses to water availability, relative to the extreme seasonal event. In our study, the seed bank of the exotic *U. humidicola* was present in both seasonal periods, and in every year, raising evidences that it forms a persistent bank to survive to extreme seasonal events. In addition, the observed higher germination post-flood asserts our hypothesis that the grass can use as a colonization strategy the germination after the extreme seasonal event, when conditions for seedling establishment become favorable.

Exotic species such as of the genus *Urochloa* to be sold shall have various pre-germinated requirements to overcome dormancy and become permeable (Marcos Filho et al. 1987). In plant communities of wet zones the specific requirements of dormancy break and of germination in some cases are favored by the environment (Baskin and Baskin 2014). The highest germination in the post-flood period found in our study can be due to softening of the tegument (in *Urochloa*, palea and lemma), that facilitates soaking and promotes breaking dormancy by an environmental physical action (Kozłowski 1997). Seed survival to flood reveals the evolutive success in adaptation of this exotic species in the floodable grasslands of the Pantanal. Several studies with grasses in the Pantanal show that the greatest germination is

linked to the end of the flood period, such as in: *Digitaria fuscescens*, *D. horizontalis*, *Paspalum alatum*, *P. plicatulum*, *Pennisetum glaucum* and *Reimarochloa acuta* (Bao et al. 2014, in the Abobral sub-region); *D. ciliaris*, *D. cuyabensis*, *Hemarthria altissima* and *Leersia hexandra* (Souza et al. 2016, in the Miranda sub-region); *Steinchisma laxum* and *Setaria parviflora* (Oliveira et al. 2015, in the Barão do Melgaço sub-region).

The post-dry period also showed germination of *U. humidicola* seeds, revealing build-up of a seed bank in both seasonal periods. Despite the formation of a seed bank during drought, this was in a lower density, but the success of colonization of *U. humidicola* in this period is attributed to vegetative spread (Soares Filho 1994). Increase of cover (ca. 46.3%) by regrowth in the dry period had already been observed (Bao et al. 2015). The high propagation potential of exotic grasses, such as *U. humidicola*, is characterized for being strongly stoloniferous (Valle et al. 2015).

The germination of *U. humidicola* in both periods (dry and flooded) indicates that in greenhouse (under controlled soil moisture conditions) this species demonstrates the power to form a bank of persistent seeds capable of germinating throughout the year. The germination density increase curve over the months reflects the importance of evaluating the seed bank of a perennial species for one year, since the germination requirements are variable between species (Baker 1989). The longevity of the perennial seed bank is attributed to environmental and storage conditions (Leck 1989). When the seeds were placed under controlled conditions in greenhouse, the seeds of both periods, had the highest germination between 5<sup>th</sup>, 6<sup>th</sup> and 7<sup>th</sup> months. In the field, it has been reported that there is no germination of new seedlings during flooding (Bao et al. 2015). This fact may be the key to controlling exotic species with invasive characteristics, regardless of their ability to propagate and regrow after the disturbance (Kelly and Parker 1990). In this way, we can predict that long periods of flooding in the Pantanal can act in a positive way, avoiding the germination of exotic species and favoring the native species adapted to these flood conditions.

The study of the *U. humidicola* seed bank was the first step to predict how the native plant community will respond in natural regeneration processes during and after the extreme seasonal event, with this data we can predict the average *U. humidicola* seed stock in soil and that is the ideal period of control of the seed bank. Our hypotheses were supported, with evidence that flooding may act as a stimulus for *U. humidicola* seed germination and that germination may occur at high density over the months under suitable conditions.

Evaluation of the tolerance to flooding time and effect of coexistence with aquatic macrophytes

Seasonal extreme events are widely known to affect community composition (Collins et al. 1995), about everything in pasture areas (Smith and Knapp 1999), because they are linked to the input of invasive species, which are the main cause of declines of native species (Bauer 2012). However, the results we have seen in our study through the association of flooding with the presence of aquatic macrophytes indicate that the extreme seasonal event is also important in our system. Independent on the high flood tolerance of *U. humidicola*, as seen in morphophysiological tests (Dias-Filho and Carvalho 2000). Native pastures can be maintained due to annual floods, since we observed a reduction in aerial cover in ca. of 70% of *U. humidicola* seedlings in the presence of floating aquatic macrophytes. In the field, this reduction had already been observed in post-flood periods (Bao et al. 2015), when many species of aquatic plants and amphibians germinate (Pott and Silva 2015), which are present in the high seed bank richness (Bao et al. 2014; Oliveira et al. 2015; Pagotto et al. 2011; Souza et al. 2016), these seed banks may be responsible for the control of exotic grass.

The high mortality of *U. humidicola* induced by floating aquatic macrophytes may be associated with decreased light and oxygen, which stress and kill many exotic species, even those with dominance characteristics (Blake and Duffy 2012). Other authors reported loss of *Urochloa* aerial part when flooded (Baruch 1994; Chapman and Lemaire 1993), and due to shading (Dias-Filho and Carvalho 2000; Mattos et al. 2005), which promotes nutrient

distribution in the leaves (Trought and Drew 1980). However, *U. humidicola* could survive under running water, such as seasonal streams and riparian zones, with more oxygen and less aquatic macrophytes with floating leaves.

Our finding indicates that if the adult population of *U. humidicola* is hampered by the occurrence of aquatic macrophytes during flooding, it can be eliminated. But the regrowth mechanism must be controlled (Neilson et al. 2005), as well as the seed bank as reported in this study. However, habitats prone to seasonal variation present changes in the plant community every year, so knowledge about the mechanisms of germination and colonization becomes fundamental for the control of exotic species (Middleton 2003), thereby the existence of gaps in their adaptation to environmental change can be the key to the restoration and conservation of native flora.

This experimental study may contribute to the understanding of some points of interest that were previously not perceptible in the field, this experiment associated with field studies complements and contributes to plans for restoration and conservation of native flora. Thus, our experimental results provide an estimate of the seed density of the exotic grass present in the soil and reinforce evidence that, after extreme seasonal events, alongside its potential for repopulation, the species has a seed bank capable of germinating and colonizing these grasslands. In addition, our data reveal the flood tolerance of the species and give us information that the existence of exotic species may be limited by the timely shading of aquatic macrophytes during floods. Thus, it is possible to analyse whether a seed addition of native species during and after flooding would be sufficient to control the propagation of *U. humidicola* in the grassland.

### **Acknowledgements**

The authors thank to INAU (Instituto Nacional de Ciência e Tecnologia de Áreas Úmidas, or “Brazilian Institute for Wetlands”), for support and finance of the project, and to the Brazilian governmental agency CNPq (Conselho Nacional de Desenvolvimento Científico e

Tecnológico, or "National Counsel of Technological and Scientific Development") for the scholarship (F.Bao) and research grants (A.P. and M.A.A.).

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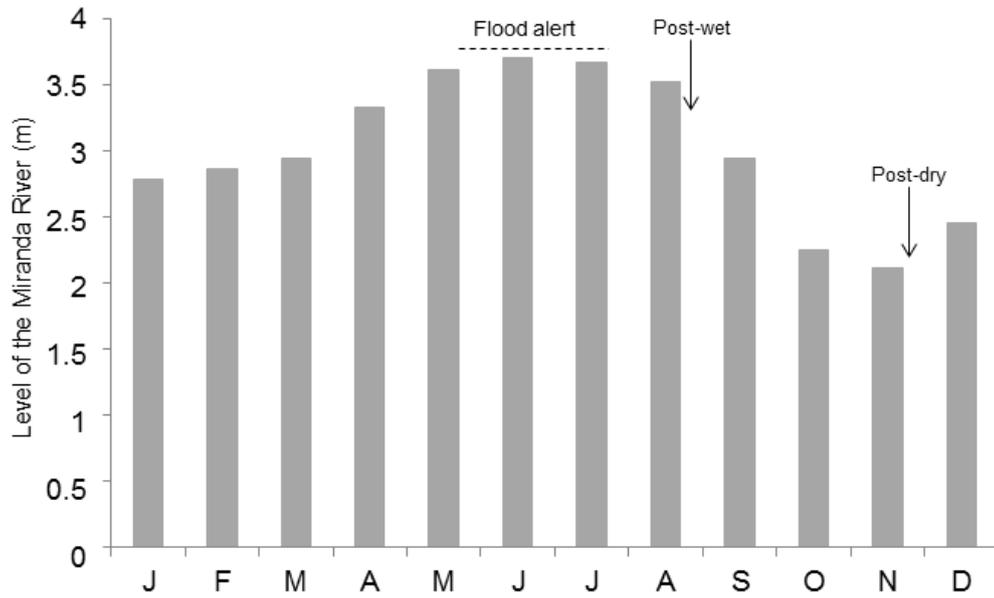
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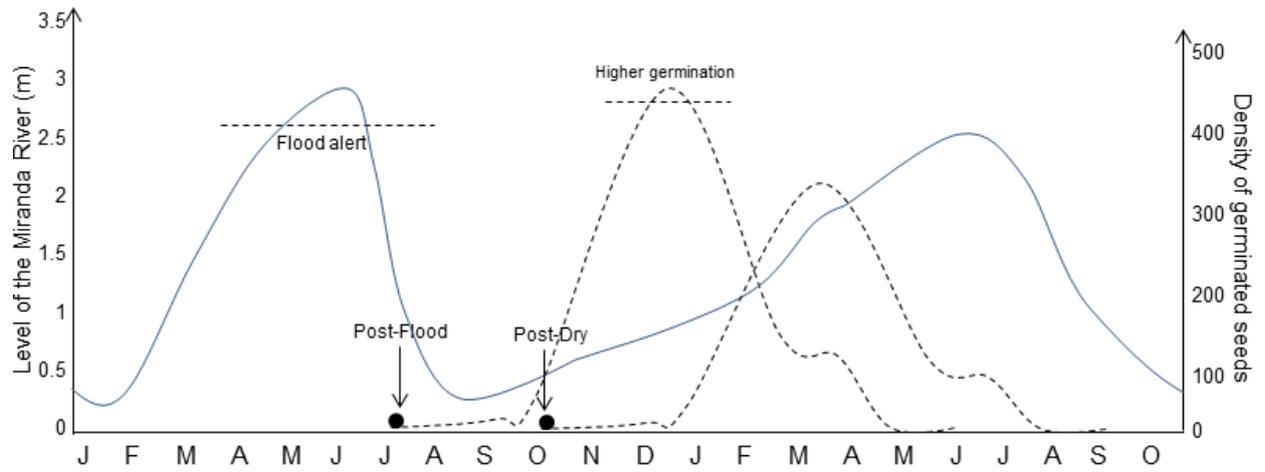
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## **Supplementary Material**



**Fig S1** Mean monthly level of the Miranda River, Mato Grosso do Sul, Brazil, arrows showing the sampled seasonal periods (post-dry and post-wet), between the years 2013, 2014 and 2015.



**Fig S2** Illustrative picture of the relationship between the mean of the Miranda river (m) in relation to the germination data of *U. humidicola* (total density) during post-flood and post-dry in grassland.

## Capítulo III

# **Seed bank of seasonally flooded grassland: experimental simulation of flood and post-flood**

Francielli Bao

Tracy Elsey-Quirk, Marco Antonio de Assis, Arnildo Pott

### **Abstract**

Wetland seed banks comprise the propagules of plant species that have species-specific germination requirements for germination in either flooded or dry conditions. At the community level, wetland structure and succession during and after a seasonal flooding event depends upon the early life history requirements of species, including germination under flooded and dry conditions. We examined the effects of simulated flood and post-flood scenarios on seedling emergence from a seed bank of seasonally flooded grassland in the Pantanal, Brazil. Field samplings were conducted in both wet and dry seasons, both of which were subject to flood and post-flood conditions. A total of 70 species emerged from the seed bank, dominated by Poaceae and Cyperaceae. Sixteen species were exclusive to the wet and one exclusive to the dry season. The richness of perennial species was higher under flood conditions, while the richness of annuals was greater post-flood. In general, the aquatic and amphibious species exhibited a significant germination response to flooding. Terrestrial species only germinated in post-flood conditions, with higher richness in the dry season. Four species had high seedling abundance in both treatments. The capacity of regeneration by seeds is high in these grasslands and can be increased by seasonal flooding and drawdown. In these seasonally flooded grasslands, we observed three main germination strategies: under flooded conditions, aquatic and amphibious species; post-flood conditions, an explosion of annual amphibious and terrestrial species; in moist soil, perennial terrestrial species. The differential responses to flooding *vs.* post-flood conditions helps to maintain the structure and species richness in the community over time.

**Keywords:** growth forms; life cycle; seasonal flooding; seedling, wetland.

## **Introduction**

Seasonally flooded wetlands can harbor a mixture of plant species tolerant of flooded and/or dry conditions (Grime 1977). Germination strategies are often cued to changes in soil moisture such that a different suite of species germinates in flooded versus dry conditions (Baskin and Baskin 2014). Across time, the richness and diversity of plant communities in seasonally flooded wetlands is dependent on both flooding and drawdown to allow the conditions necessary for germination and growth of individual species (Bonis et al. 1995; Pott and Pott 2004; Pott et al. 2011).

During flood periods, aquatic species may dominate, while during dry seasons, more terrestrial species may prevail. During each of the seasonal conditions, a group of species (aquatic/terrestrial) will be regenerating (Nielsen et al. 2002), that is, in seasonal wetlands the germination of many species varies according to the amount of water present in the soil (Brock 2011). Seasonal wetlands dominated by herbaceous species present an ideal system for examining regeneration strategies, due to the existence of two stable but alternating annual seasons (Ames et al. 2015) and rapid colonization of annual species (Barton et al. 2016), giving an answer in a short time.

The replacement of species in floodable grasslands can be limited by the availability of seeds (or other propagules) and/or of seedling establishment. Seed limitation occurs when not all seeds reach available sites for germination (Hubbell and Foster 1986). This can be due to the number of seeds (in higher number - annual plants; fewer - perennials) and/or limited seed dispersal (Schneider 1994; Dalling et al. 1998). In flood periods, corridors are formed that carry seeds for potentially long distances (Middleton et al. 2003). Seeds can be trapped in the soil in different densities according to the topographic gradient (Pagotto et al. 2011; Bao et al. 2014). This environment can limit species richness and abundance in the community (Muller-Landau et al. 2002). Yet the limitation of establishment occurs when not all seeds germinate. Such limitation will depend on germination itself, with a direct influence by environmental factors

including moisture, light, temperature (Long et al. 2015), and effect of seasonality (Hölzel and Otte 2001).

The germination response to seasonal events varies according to the duration and intensity of the flood, determining the occurrence or not of different groups of species (aquatic, amphibian and terrestrial) this is a fundamental mechanism for the structuring and composition of a plant community (Jutila 2001). The Brazilian Pantanal is marked by predictable annual floods (Pott and Silva 2015), where grasslands are flooded approximately three to five months per year. The magnitude of flooding, however, varies with topography and annual rainfall (the latter influenced, for example, by El Niño events) (Junk 1993; Pott and Pott 2004). Flooding of the grassland stimulates germination of aquatic macrophytes (Oliveira et al. 2015; Souza et al. 2016), while the seeds of terrestrial plants adapted to drier conditions remain in the seed bank waiting for the ideal conditions for colonization (Bao et al. 2014).

The evaluation of the seed bank of wetlands can provide us with information on the different plant life-forms involved in the regeneration of this plant community under different environmental conditions, in order to identify which plant species can establish during and after a seasonal flood event (LaDeua e Ellison 1999; LePage et al., 2000; Brock 2011), and the species available for regeneration. These are important data to help inform understanding of factors influencing the continued survival of wetland plant communities, and to aid design strategies for their conservation (Thompson et al., 1997). Our study also provides new information on how differing species germination strategies, and variation in seed flood tolerance, may influence plant community succession in seasonally-flooded tropical wetlands.

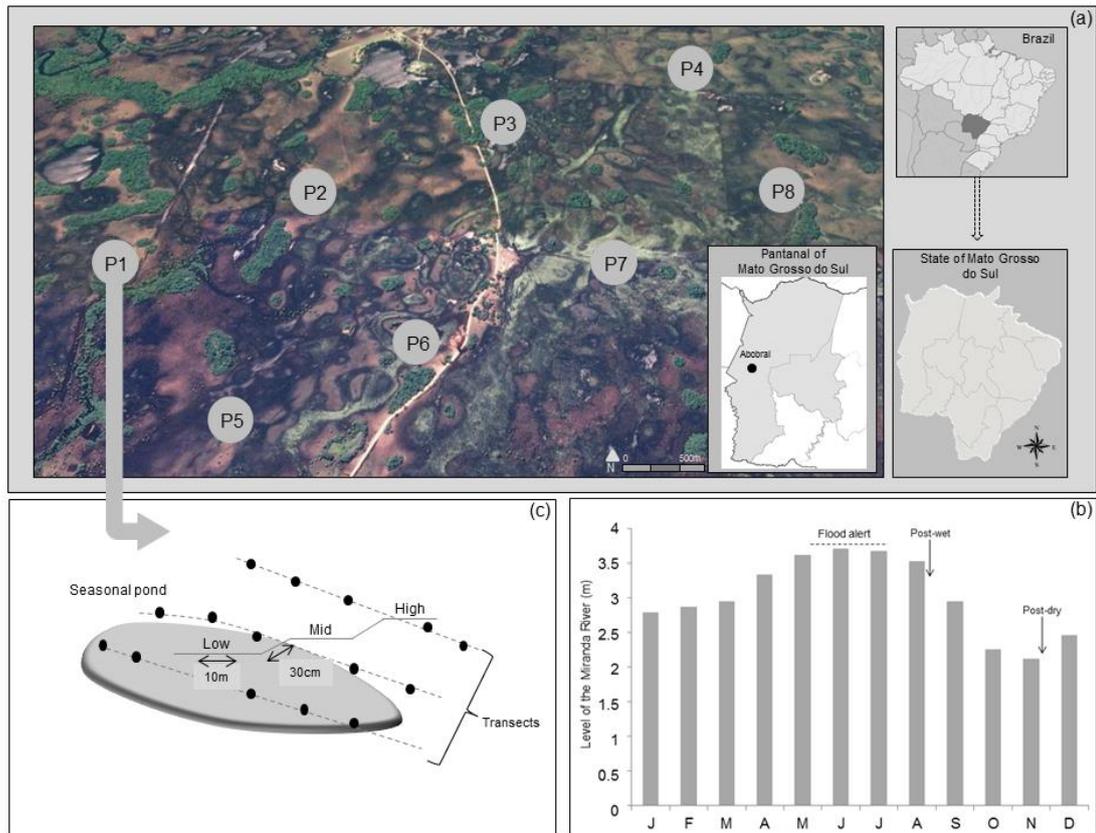
Our objectives were to evaluate the seed bank of floodable grasslands and to investigate how the life forms of the communities of species present in these dynamic systems respond to seasonal floods. We carried out experiments to simulate the effect of flood and post-flood conditions on the seed bank to examine changes in emergence from the seed bank. Specifically, we examined: (i) species primarily involved in initial regeneration during and after flooded and

dry conditions; (ii) life history regenerative strategies in the seed bank in relation to seasonal variation (cycle of annual and perennial species); and (iii) the established species in the grassland during flood and post-flood conditions (contrasting the occurrence of aquatic, amphibious and terrestrial species).

## **Methods**

### Region of study

The Brazilian Pantanal wetland is the largest continuous floodplain on Earth, covering 140,000 km<sup>2</sup>, in Central-West Brazil, predominantly occupied by seasonally flooded grasslands and savannas. Flooding in the Pantanal is annual and predictable (Pott and Silva 2015) due to local rains and/or river overflow (Pott and Pott 2004). Topographic variation combined with local hydrologic regimes form permanently and periodically flooded zones (Pott and Silva 2015). The climate is tropical sub-humid, with an average annual rainfall of 1100 mm, and mean annual temperature of 26°C (Allem and Valls 1987; Silva and Abdon 1998). The soil is sandy. The study area in Central-West Brazil (Fig. 1a), covering 9200 ha, is a seasonally flooded plain with natural grasslands and temporary water bodies containing aquatic macrophytes and, in the dry areas, numerous grasses and terrestrial herbs (Pott and Pott 2004).



**Fig 1** Seasonally flooded grassland in the Pantanal wetland (Central-West Brazil). **(a)** Sampling points including eight seasonal ponds (P1-P8 in grassland - Image Source: “Fazenda São Bento”, south Pantanal, (19°29’27.3”S; 57°01’55.9”W) Google Earth (accessed 15 March 2017)), **(b)** mean monthly level of the Miranda River, arrows showing the sampled seasonal periods (dry and wet), between the years 2005-2015, **(c)** representation of the transects following the topographic levels (low, mid and high) in each sampled pond, with five random samples.

The study area is utilized as pasture, mostly comprised of native grasses, but approximately 20% of it was cultivated with the exotic *Urochloa humidicola* (Rendle) Morrone & Zuloaga. Flooding occurs between April and August with maximum water levels approximately 4 m above baseline datum (a.b.d.) in the nearby Miranda River in June-July and minimum water levels of 2 m (a.b.d.) typically between October and December level (data collected at ranch Fazenda São Bento, between 2013 and 2015, Fig.1b). Therefore, the floodplain undergoes predictable seasonal cycles of flooding and drawdown.

### Seed bank collection

To evaluate the effect of seasonal flooding on seed bank composition, we sampled three elevation levels of eight temporary ponds during two periods characteristic of the Pantanal: the end of the wet season in July and August (2014 and 2015), and after the dry season in October and November (2013 and 2014). Ponds were selected with similar depths and areas. At each pond, three 10m long transects were established along a topographic gradient. Topographic levels (e.g., low, mid, and high) with approximately 30 cm vertical difference were established according to a reference of the flood-level water mark on fence poles and plants (Fig. 1c). Low topographic level was in the middle of the pond, the Mid level was on the pond edge, and the High level was on less flooded, higher ground. Stratifying by topographic level allowed us to sample the major habitat types, which may be structuring the seed bank and plant community in this system (e.g., Bao et al. 2014, Bao et al. 2015), during different densities of seeds captured at each level, due to the lowering of the waters after the seasonal flood (Pagotto et al., 2011; Bao et al., 2014). At Low topographic levels, there is a higher seed density and a higher concentration of seeds of aquatic macrophytes. At the Mid and High levels, the number of seeds of grasses and seeds of terrestrial grass increases (Bao et al., 2014).

Five soil samples were collected randomly along each transect according to a table of random numbers. The upper 3 cm depth of soil sample was collected within 20 x 20 cm quadrats. Soil samples were kept in dark plastic bags, under ambient temperature and transported to Universidade Federal de Mato Grosso do Sul (UFMS), immediately for germination studies.

### Seed bank experiments

Soil samples were spread in plastic trays (20 x 20 x 10 cm) and then subject to a two-phase flooding regime simulating a three month flood followed by a three month drawdown. Specifically, samples were first submerged in tanks under 90 cm of water (flood treatment) for

three months, the flood in tanks was similar to the depth observed in the field. Any filamentous algae growth was removed. Seedlings of aquatic plants were removed and placed into trays with bare soil in the greenhouse (post-flood treatment). Here, they were exposed to ambient air temperature and twice a day watering for three months. Samples were randomly placed within the greenhouse and were moved around monthly to provide further randomization of light conditions (e.g., Bao et al. 2014).

The seed bank was evaluated using the seedling emergence method, whereby germinated seeds were counted, identified and removed to avoid competition with new seedlings (Thompson et al. 1997) once per week. Species identification of some species was only possible during flowering (e.g., Pott and Pott 1994, Pott and Pott 2000). Vouchers of all species were incorporated in the Herbarium CGMS, at the Universidade Federal de Mato Grosso do Sul. Nomenclature follows APG IV-Angiosperm Phylogeny Group (2016). Species germinated from the seed bank were classified according to their life cycle (perennial or annual), growth form (aquatic, terrestrial and amphibious), and whether they germinate and establish in a dry or flood environment (Pott and Pott 1994; Leck and Brock 2000; Pott and Pott 2000).

### Statistical analysis

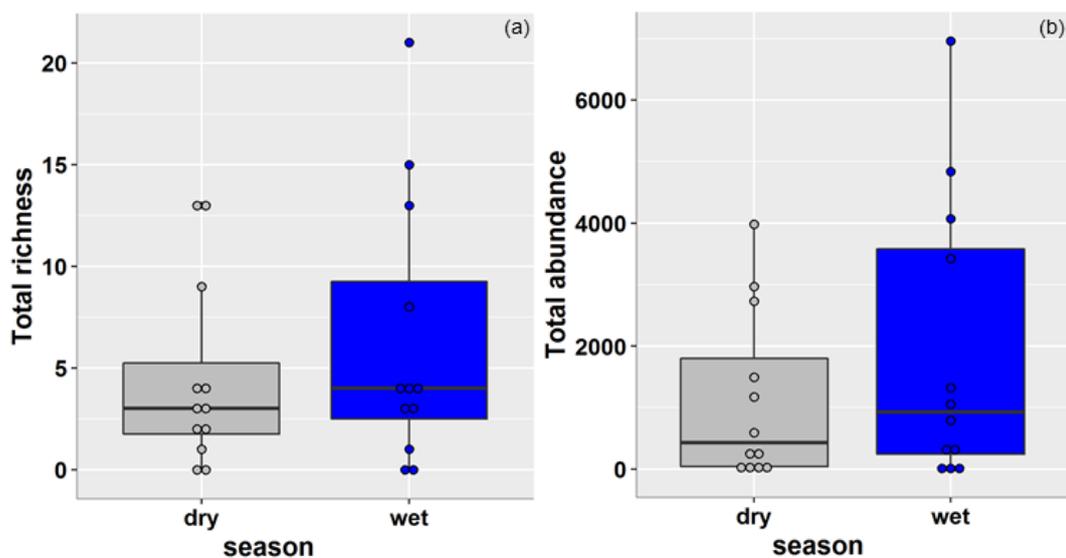
First, we constructed species accumulation curves to evaluate seed bank sampling in two treatments: flood and post-flood, and in the wet and dry seasons. Species richness and abundance data fit a Poisson distribution. Generalized linear models (GLM) (Cai et al. 2012) were used to test the effect of seasonality (wet and dry) and treatment (flood and post-flood) on species richness and abundance, as well as the interaction of treatments and seasonality. The influence of these factors on the germination of different life history strategies and growth forms was analysed separately for each seasonal period. We conducted a GLM for the species that emerged in the two treatments (flood and post-flood) and in the two periods (wet and dry season). All analyses were conducted in R environment (R Core Team 2017), with *vegan* (Oksanen et al.

2017), permute (Simpson 2016), lattice (Deepayan 2008) and BiodiversityR (Kindt and Coe 2005).

## Results

### Richness and abundance of the plant community in the seed bank

The seed bank was composed of a total of 70 species, representing 21 families and 49 genera. A full list of species and authorities is given as Table S1 in the supplementary material attached to their online version of this paper. Sixteen species were exclusive to the wet season and one species was exclusive to the dry season. Species richness during the wet season was 66 species, which was greater than during the dry season (51 species) ( $z$ -ratio = 4.569,  $p < 0.001$ , Fig. 2a). Poaceae and Cyperaceae had the highest number of species (11 species each), followed by Plantaginaceae (7 species), Euphorbiaceae (6), and Alismataceae and Asteraceae (5 species each). Seedling abundance was almost double in the wet season (23134 seeds compared to the dry season (13514 seeds) ( $z$ -ratio = 1.0489,  $p < 0.001$ , Fig. 2b).



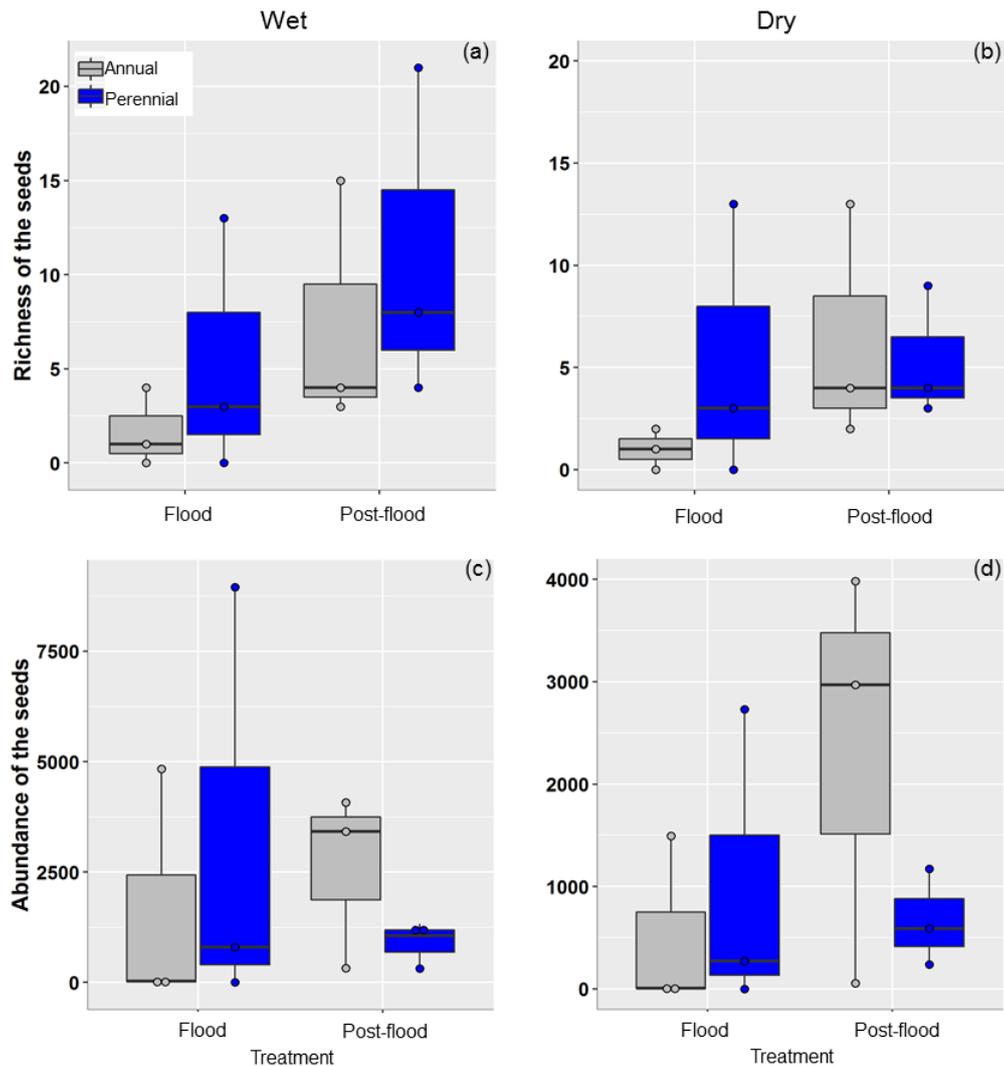
**Fig 2** (a) species richness and (b) abundance of seeds present in the seasonally flooded grassland seed bank in the Pantanal wetland (Central-West Brazil).

The species exclusive to the flood treatment were essentially aquatic plants, with the highest abundances of *Sagittaria guayanensis* (1703 seedlings), *Limnocharis flava* (1479 seedlings) and *Hydrocleys parviflora* (1292 seedlings). However, *Isoetes pedersenii* (a small fern-ally) was also found in the seed bank, with an abundance of 1247 germinated spores limited to the flood treatment (Table S1).

The species accumulation curves suggest that most species in the community were sampled (Fig. S1). This result is important because it provides support for the importance of evaluating the seed bank both flood and post-flood treatments, conditions.

#### Effect of flood and post-flood on germination of perennial and annual species in the seed bank

In the flood treatment, 32 perennial and 6 annual species germinated. In the wet season, the number of annual and perennial species in the seed bank was similar (z-ratio = 2.270,  $p=0.581$ , Fig. 3a), whereas in the dry season, a significant difference occurred between annual and perennial species (z-ratio = 2.661,  $p<0.001$ , Fig. 3b). In the post-flood treatment, the number of annual species (25 species) increased in relation to flood; but the number of perennials (32) was similar between flood and post-flood treatments. Ultimately, there was no difference in the number of annual and perennial species in the post-flood treatment for both sampling seasons.

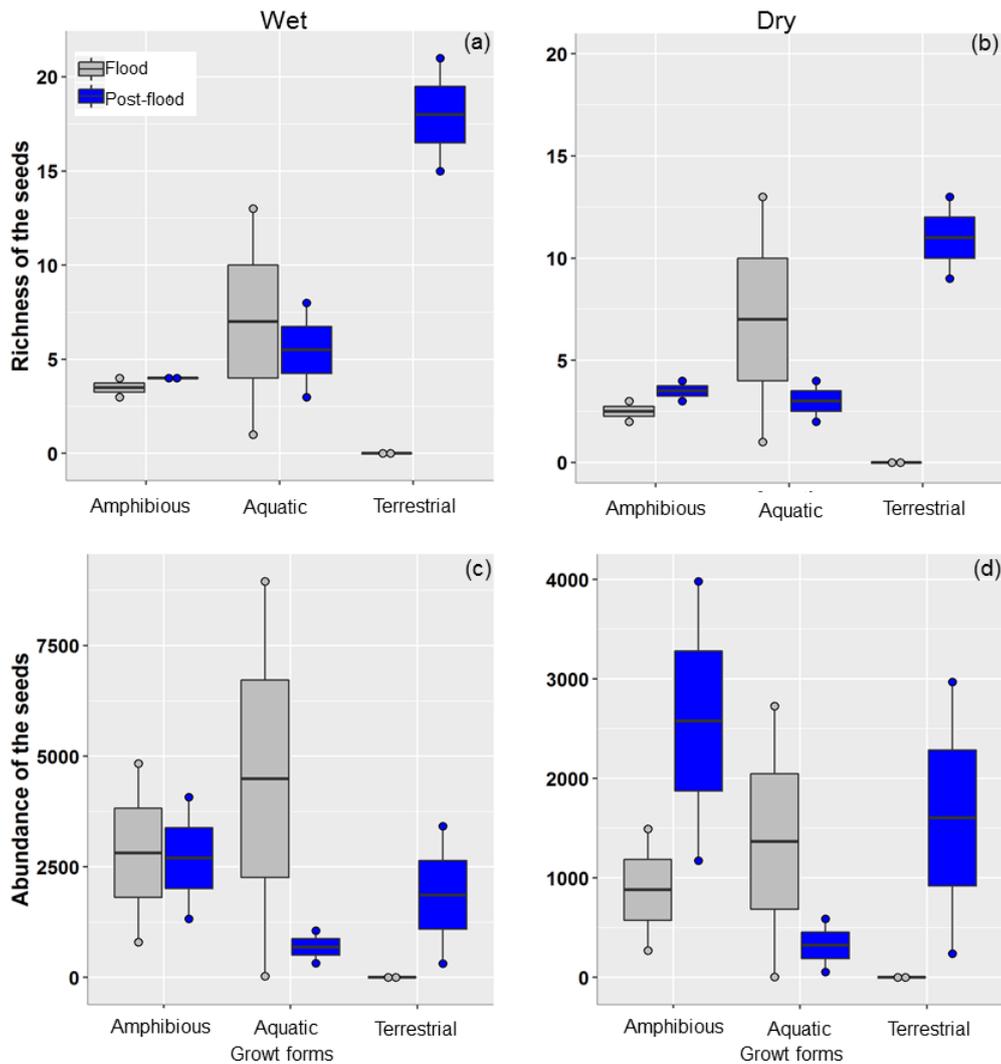


**Fig 3** Germination of annual and perennial species. Mean richness of germinated seeds (a) in the wet and (b) dry season. Mean abundance of germinated seeds, (c) in the wet and (d) dry season, between the treatments of flood and post-flood, from seed bank of seasonally flooded grasslands in the Pantanal wetland (Central-West Brazil).

Similar abundances of annual and perennial species occurred in the wet versus dry season ( $z$ -ratio = 0.574,  $p=0.581$ ;  $z$ -ratio = 0.436,  $p=0.675$ , respectively, Fig. 3c,d), however, in both the wet and dry seasons, the abundance of annual species was greater than perennial species in the post-flood treatment (wet season: 4378 annuals and 1654 perennials,  $z$ -ratio = 0.436,  $p<0.001$ , and dry season: 4582 annuals and 1769 perennials,  $z$ -ratio = 1.417,  $p<0.001$ , Fig. 3c,d).

### Interaction of seasonal collection and germination environment on growth forms

Seasonality (wet vs. dry) and germination environment (flood and post-flood) interacted to affect the richness and abundance of different growth forms. Species richness of aquatic macrophytes increased in flooded conditions, in both wet and dry sampling seasons (Table S1; wet: z-ratio = 1.497,  $p < 0.001$ , and dry season (z-ratio = 1.976,  $p < 0.001$ , Fig. 4a,b). The abundance of aquatic macrophytes was three times greater in the wet season (8534 seedlings) than in the dry season (2326 seedlings) with the greatest abundance in the flood treatment across sampling periods (wet: z-ratio = 1.976,  $p < 0.001$ , dry: z-ratio = 0.393,  $p < 0.001$ , Fig. 4c,d). Terrestrial plant species only germinated in the post-flood treatment, with a richness of 38 species in the wet season and 23 in the dry season. Abundance of terrestrial species was similar in the wet and dry seasons. Richness of amphibious plants did not differ between seasonal collections or germination treatment (Fig. 4c,d; Table S1); however there was a greater abundance of amphibious species that germinated from samples collected in the wet season than in the dry.



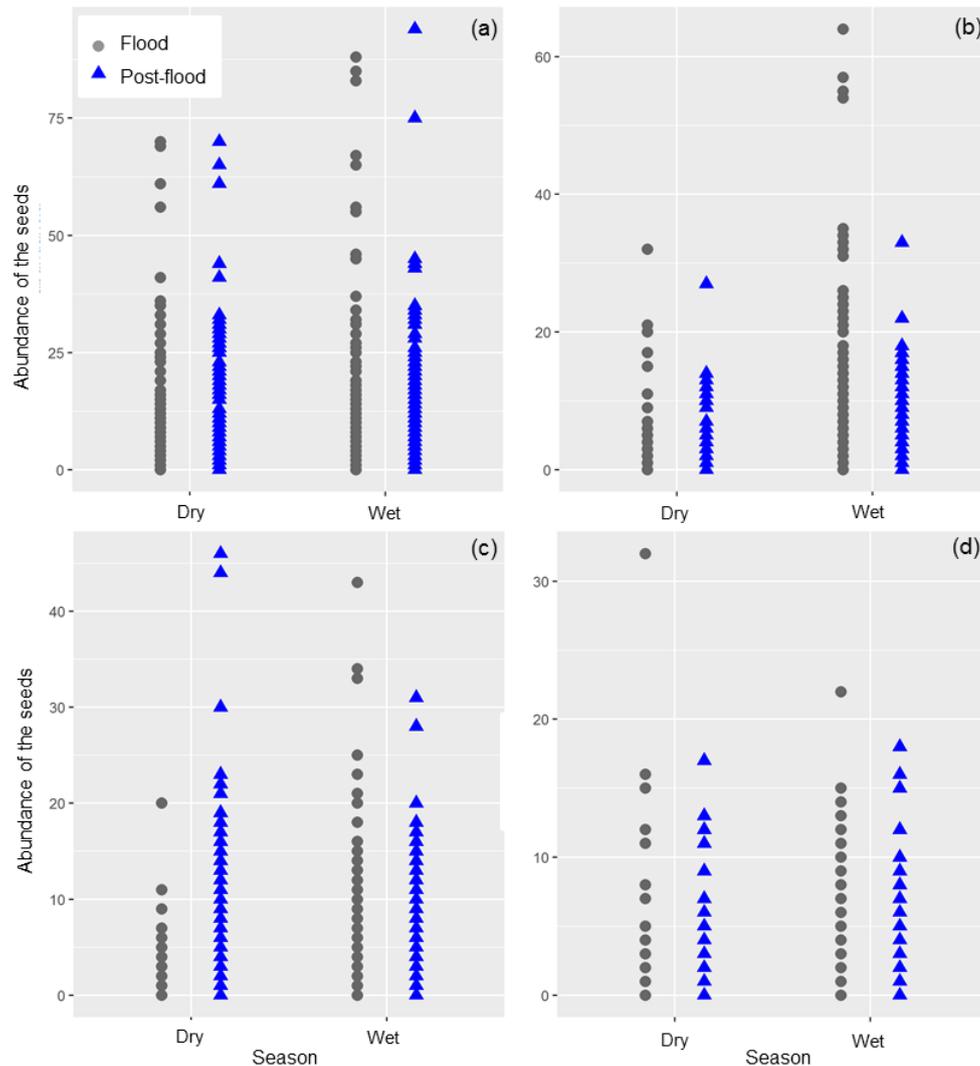
**Fig 4** Effect of treatments of flood and post-flood on species richness (a) in the wet and (b) dry season; and on seed abundance, (c) in the wet and (d) dry season, between the different growth forms, in seasonally flooded grasslands in the Pantanal wetland (Central-West Brazil).

Yet, in the dry season seed germination of amphibious species was three times higher in the post-flood treatment than in the flood treatment ( $z$ -ratio = 7.872,  $p < 0.001$ , Fig. 4d). Four species occurred in all treatments and in all sampling seasons, and had the greatest abundance of germinated seeds: *Rotala ramosior* (8989 seedlings), *Eleocharis minima* (3826), *Bacopa australis* (3581) and *Helanthisum tenellum* (1564) (Table 1).

**Table 1** Number of emerged seedlings in the treatments flood and post-flood of four species shared between two seasonal periods (dry and wet), from the soil seed bank of seasonally flooded grassland in the Pantanal wetland (Central-West Brazil).

Species	Flood	Post-Flood	Estimate	Mean	SE	Pr(> t )	t value
<i>Rotala ramosior</i>	4854	4135	0.1603	1.8516	0.0907	0.0777	1.7666
<i>Bacopa australis</i>	2350	1231	0.6466	1.2495	0.1107	<b>0.0044</b>	2.849
<i>Helanthium tenellum</i>	914	650	0.3408	1.2512	0.1316	<b>0.0097</b>	2.590
<i>Eleocharis minima</i>	1631	2195	0.2970	1.8986	0.1021	<b>0.0037</b>	2.910

In the individual analysis of each species, *R. ramosior* had similar germination in flood versus post-flood conditions, however, germination of the other species differed between treatments (Fig. 5). Only *E. minima* had higher germination in the post-flood treatment (Table 1). Yet, these species had higher germination from soil in the wet season than the dry season: *R. ramosior* (z-ratio = 5.774,  $p < 0.001$ , Fig. 5a), *B. australis* (z-ratio = 11.738,  $p < 0.001$ , Fig. 5b), *E. minima* (z-ratio = 5.544,  $p < 0.001$ , 5c) and *H. tenellum* (z-ratio = 6.292,  $p < 0.001$ , Fig. 5d).



**Fig 5** Total abundance of emerged seedlings of four shared species under the treatments flood and post-flood: (a) *Rotala ramosior*, (b) *Bacopa australis*, (c) *Eleocharis minima* and (d) *Helanthis tenellum*, from the soil seed bank of two seasonal periods (wet and dry) of floodable grasslands in the Pantanal wetland (Central-West Brazil).

## Discussion

Regeneration of seasonally flooded grasslands, such as those of the Pantanal, occurs through the high regeneration capacity of annual and perennial species (Zobel et al. 1998; Geerthemia et al. 2002), which have distinct germination strategies, varying according to the environmental conditions (Wenzhi et al. 2009), and the abundance of annual species in the seed bank (Bao et al. 2014; Souza et al. 2016). In our study, three strategies/traits of germination were

found in the seed bank of floodable grasslands in the Pantanal, according to soil flooding and drought.

#### Richness and abundance of the plant community in the seed bank

The abundance and richness of the seed bank varied between the treatments of flood and post-flood. The treatment with soil inundation revealed a seed bank full of aquatic macrophytes (Fig. 2S). Nevertheless, similar to other reports (van der Valk and Davis 1978; Leck and Simpson 1987; Boedeltje et al. 2002), there was a higher species richness and abundance of seedlings from non-inundated soil than submerged soil. In spite of the low species richness in flood conditions, perennial species were very abundant. Initially, emergence of *Rotala ramosior* and *Bacopa australis* was high followed by mostly aquatic species such as *Hydrocleys parviflora*, *Limnocharis flava* and *Sagittaria guayanensis*, and plants commonly found in temporary ponds, such as *Pontederia parviflora*, *Echinodorus grandiflorus* and *Helanthium tenellum* (Pott and Pott 2004).

High species richness of Poaceae and Cyperaceae is characteristic of seasonal savannas, where aquatic and terrestrial phases alternate, with predominance of the grasses *Reimarochloa* spp., *Axonopus purpusii*, *Paspalum* spp. (Pott and Pott 1997, Pott and Pott 2004). In the seed bank, we also found *Digitaria bicornis*, *D. fuscescens* and *Setaria parviflora* in high abundance. Under increased moisture, the grassland is filled with *Cyperus* spp. and *Eleocharis* spp. (Pott and Pott 2004). All recorded species are present in the list of flora of the Pantanal (Pott and Pott 1999).

The high occurrence of spores of *Isoetes perdersenii* in the seed bank was also reported in flooded grasslands in the northern Pantanal (Oliveira et al. 2015), where it represented over 50% of the diaspores present in the soil. The presence of spores in the soil in both seasons, is in contrast to a previous report by Bao et al. (2015), who observed the occurrence of this fern-ally

species only in the wet season. Based on our observations of spores in both seasons, we hypothesize that this species may form a persistent bank of propagules.

The species accumulation curves illustrated the importance of sampling wetlands in two seasonal periods (wet and dry) and evaluating the seed bank under both flood and post-flood conditions to maximize the representation of seeds and seedling emergence strategies. Seasonal and flooded and drained germination conditions can improve estimates of richness of the vegetation (e.g., van der Valk and Davis 1978, 1979; Bonis et al. 1995; Smolders et al. 1995; Brock 1997, 2011; Boedeltje et al. 2002; Price et al. 2010), in addition to reflecting the ecologically active species present in the seed bank (Haag 1983), particularly for species producing dormant seeds (van der Valk and Davis 1978; Bonis et al. 1995). This bias can make a considerable difference, since, in our study, the germination rate under experimental flood conditions increased the species abundance and richness of the plant community. Without the flood conditions, aquatic species would have been neglected. This information has enhanced our understanding of seed banks in these flooded grasslands, as previous studies of the seed bank in the Pantanal did not evaluate the flood conditions (e.g., Pagotto et al. 2011; Bao et al. 2014).

Effect of flood and post-flood conditions on germination of perennial and annual species in the seed bank

Greater richness of perennial species in the seed bank in flooded conditions reveals that many species of temporarily wet zones form seed banks, rich in perennial species (Fig. 2S). It is known that the seed bank of perennials is in general persistent (Thompson and Grime 1979), due to the dormancy of most species of this group. However, the germination strategies of many species are limited by environmental conditions (Saatkamp et al. 2014), and some species may change their strategies according to environmental variation. Yet, the low richness of annual plants in relation to perennials, compared with other wetlands (e.g., Skoglund and Hytteborn 1990; Leck and Brock 2000), can be due to our experimental conditions that favored the

germination of many aquatic plants and perennials. To the knowledge of the seeds that make up the bank and that are potentially able to regenerate these pasture fields during and after the extreme seasonal event.

In wetlands, where the environment is variable, reproduction can fail in some consecutive years, which makes seed banks critical for the persistence of the plant community (Bonis et al. 1995), providing a mechanism to buffer the effect of seasonal events impacting the capacity of the seed bank to regenerate these plant communities. Our data reveal that many perennial species present in the seed bank are able to colonize during and after flood disturbances, supplementing the mechanisms of regrowth and vegetative propagation otherwise characteristic of grasses and sedges (Ernst et al. 1992), characteristic of grasses and sedges.

The communities of annual and perennial plants in a variable environment undergo strong variations of abundance over time (Venable and Lawlor 1980). The variation in density of seeds in the soil depends mainly on germination and reproduction of each species (Bonis et al. 1995). In our study, the abundance of annual over perennial species, in post-flood (Fig. 3S), was due to annual species producing large quantities of seeds (Oliveira et al. 2015; Souza et al. 2016), which disperse readily (Cohen 1967; Bonis et al. 1995; Baldwin and Derico 1999). Nonetheless, it is evident that after a flood, there is an increase in emergence of annuals (Souza et al. 2016).

A higher number of seeds retained in the soil corresponding to a flood period is characteristic of seasonally flooded wetlands (e.g., in the Pantanal: Pagotto et al. 2011; Bao et al. 2014; Oliveira et al. 2015; Souza et al. 2016; in others wetlands: Bertiller 1992, Leck and Leck 1998; Leck and Brock 2000; Brock 2011), due to the increased incidence of dispersal corridors (Johansson et al. 1996; Middleton et al. 2003), and seeds being trapped in areas of lower elevation (Pagotto et al. 2011; Bao et al. 2014).

Interaction of seasonal collection and germination environment on growth forms

The coexistence of aquatic, amphibious and terrestrial plants in wetlands indicates that these grasslands are large amphibious habitats under constant succession (Pott et al. 2011). Species replacement is caused by the environmental conditions required by different growth forms for germination and growth, related to seasonal conditions of moisture, temperature and flooding in each season (Grillas et al. 1991).

We observed an interaction between growth forms and germination treatments of flood and post-flood due to the groups of aquatic and terrestrial plants having their germination restricted by the presence or absence of a flood. Some essentially aquatic species occasionally germinated following inundation, although, no terrestrial species germinated while flooded.

In these seasonally flooded savannas, the predominance of terrestrial grasses was high, but as flooding begins, numerous other terrestrial, amphibious and aquatic herbs cover the ground, followed by previously dormant aquatic macrophytes (Pott and Pott 2011). As the water recedes, the aquatic species die and form a layer of organic debris (Bonis et al. 1995) and terrestrial annual herbaceous species germinate (Bonis and Lepart 1994; Bonis et al. 1995). Thus, the similarity of the seed bank is high before and after flood (Bao et al. 2014), reflecting the seasonal dynamics of these plant communities (Casanova and Brock 1990) and the capacity to reestablish during and after an extreme seasonal flooding event (Bao et al. 2015).

Our results reveal that many aquatic plants species have high potential to colonize wetlands via the seed bank, contrary to the findings of other studies that reported colonization of such plants only when vegetative propagules such as rhizomes or bulbs are present in the soil (Brix 1994; Poschlod et al. 1999; Engelhardt and Ritchie 2001; Sosnová et al. 2010). The germination rate is related to the level of variability of the environment and to the number of seeds present in the soil (Venable and Lawlor 1980).

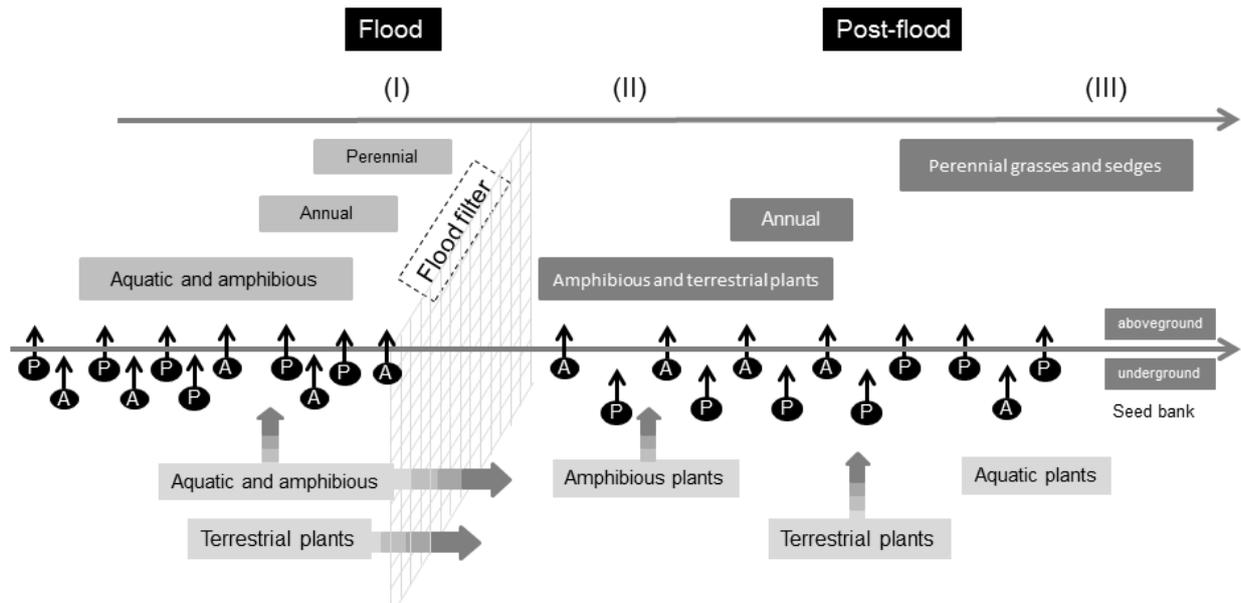
For the group of amphibious plants, we did not observe differences between the treatments and between seasons. The coexistence of amphibious and terrestrial plants is high, as well as of the amphibious and aquatic plants. However, for aquatic and terrestrial plants there

was no interaction. Germination of these groups fluctuates strongly with environmental conditions (Baskin and Baskin 2014), which allows the exclusion of species or of a group, according to the flood level (Van der Valk 1981). The seed bank introduces an effect of storage of seeds capable to colonize, either in years of flood or prolonged drought.

The seed bank seems to be formed by groups of seeds that can go extinct for a time and/or remain in the grassland constantly, independent of environmental conditions. Considering the latter, we found, our bank revealed four species with high germination rates: *R. ramosior*, *B. australis*, *H. tenellum* and *E. minima* (Fig. 3S). These small herbaceous species are consistently found in the seed banks in the Pantanal (Pagotto et al. 2011; Bao et al. 2014; Oliveira et al. 2015; Sousa et al. 2016), and in natural grasslands comma and grow intermingled with native and exotic grasses (Bao et al. 2015). They represent a group of “flexible” species that germinate and become established in the field, independent of flood/dry dynamics.

### **Conclusions and future perspectives**

This study highlights three germination strategies of species represented in the seed bank of floodable grasslands in the present study: (I) annual and perennial species of aquatic and some amphibious plants when the soil is under total inundation - (II) an explosion of annual species, with amphibious and terrestrial plants, immediately after the flood during drawdown; and (III) some annual species and numerous perennial grasses and sedges, typically terrestrial, which start germination post-flood. When the soil is flooded, most annual and some perennial grasses are excluded and conversely, when soil is no longer flooded aquatic plants (mostly perennials – many Cyperaceae) are largely excluded. Nonetheless, some species adapted to these seasonal systems can germinate and become established under either conditions (Fig. 6).



**Fig 6** General scheme of three germination strategies in our experimental study on the bank of seeds of seasonally flooded grassland: (I) when the soil is under full flood; (II) when the soil is immediately released from the flood, and (III) over the months maintaining the soil moisture.

The dynamics of flood and dry conditions in these seasonal grasslands are important, with each group of species having its germination strategy, according to the environmental conditions. Our results show that this plant community has high species richness and abundances of seeds, which contributes to the initial colonization during and after seasonal events. Climate change, leading to reduced flooding, may modify this scenario, where large groups of species may be lost if flooding declines or increases over time. In our study over two years of sampling, it was not possible to verify a temporal change in the composition of the vegetation. But it is worth noting the importance of the evaluation of the seed bank as the main source of maintenance of the plant community, mainly because it contains species of different growth forms. We also highlight the importance of evaluating seed banks in different seasons and in two phases: flood and dry, to better assess the plant diversity of seasonally flooded grasslands.

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## **Supplementary Material**

**Table S1** List of species with their respective values of abundance of germinated seeds from the seed bank under effect of the treatments of flood and post-flood, under seasonal variation (wet and dry season), classified regarding life cycle (annuals and perennials) and growth forms (aquatic, amphibious and terrestrial), in the sub-region Abobral in the Pantanal wetland, MS, Brazil.

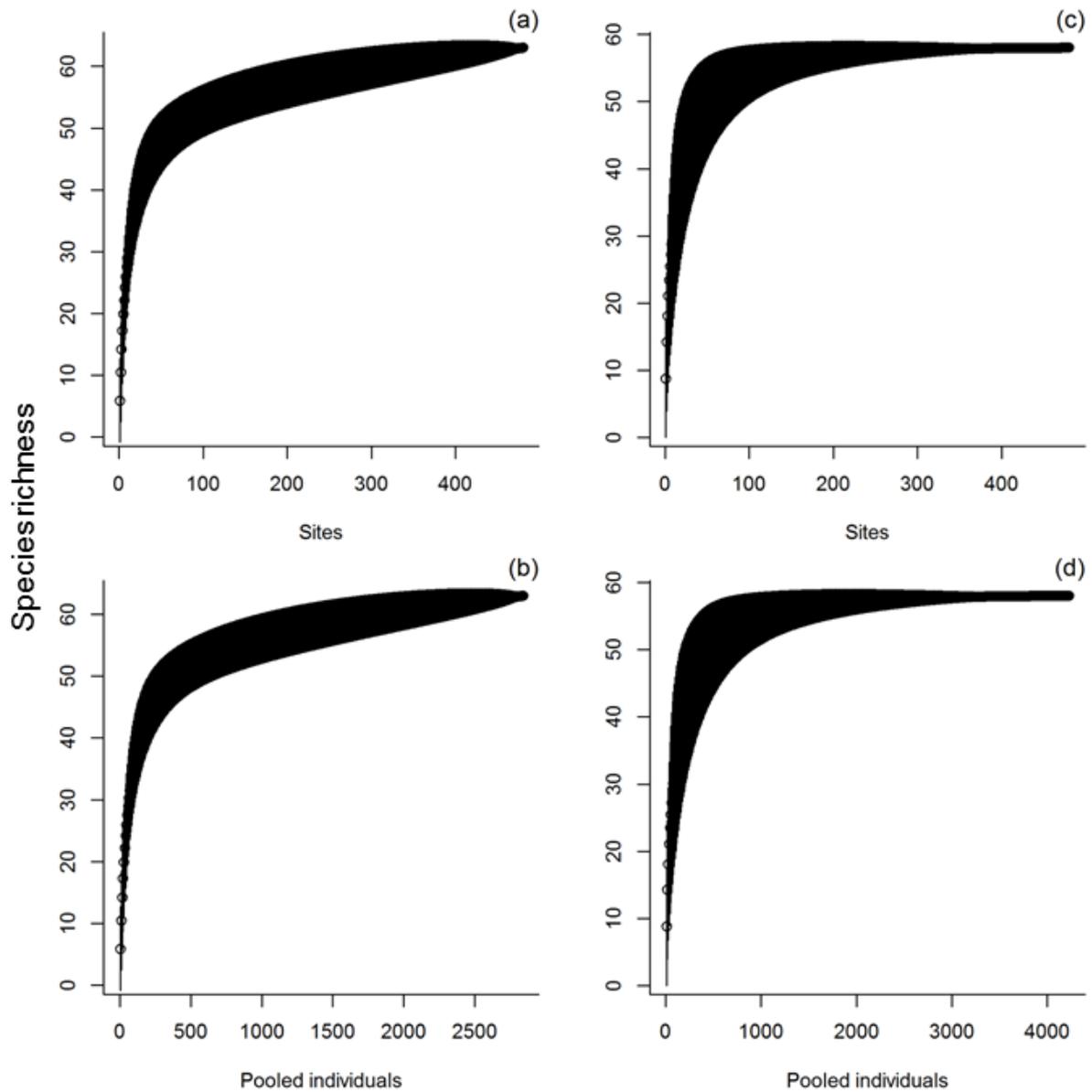
Family/species	Life cycle	Growth forms	Wet season		Dry Season	
			Post-flood	Flood	Post-flood	Flood
<b>Alismataceae</b>						
<i>Echinodorus grandiflorus</i> (Cham. & Schltld.) Micheli	P	Amphibious	18	279	0	235
<i>Helanthium tenellum</i> (Martius) Britton	P	Aquatic	319	825	269	186
<i>Hydrocleys parviflora</i> Seub.	P	Aquatic	0	1292	0	184
<i>Limnocharis flava</i> (L.) Buchenau	P	Aquatic	0	1479	0	54
<i>Sagittaria guayanensis</i> Kunth	P	Aquatic	0	1703	0	371
<b>Apiaceae</b>						
<i>Eryngium ciliatum</i> Cham. & Schltld.	A	Terrestrial	4	0	3	0
<b>Asteraceae</b>						
<i>Conyza bonariensis</i> (L.) Cronquist	A	Terrestrial	25	0	0	0
<i>Gamochaeta purpurea</i> (L.) Cabrera	P	Terrestrial	0	0	0	0
<i>Praxelis clematidea</i> R.M. King & H. Rob.	A	Terrestrial	97	0	63	0
<i>Sphagneticola brachycarpa</i> (Baker) Pruski	P	Terrestrial	42	0	35	0
<i>Stilpnopappus pantanalensis</i> H. Rob.	P	Terrestrial	33	0	41	0
<b>Cyperaceae</b>						
<i>Cyperus compressus</i> L.	P	Terrestrial	2	0	0	0
<i>C. haspan</i> L.	P	Amphibious	569	0	540	0
<i>C. lanceolatus</i> Poir.	P	Terrestrial	8	0	0	0
<i>C. surinamensis</i> Rottb.	P	Amphibious	591	0	350	0
<i>Eleocharis acutangula</i> (Roxb.) Schult.	P	Aquatic	4	346	7	36
<i>E. minima</i> Kunth	A	Amphibious	548	1521	1213	813
<i>Fimbristylis dichotoma</i> (L.) Vahl	A	Terrestrial	199	0	191	0

<i>Kyllinga brevifolia</i> Rottb.	P	Terrestrial	66	0	0	0
<i>Lipocarpa humboldtiana</i> Nees	A	Terrestrial	0	0	0	0
<i>L. micrantha</i> (Vahl) G.C.Tucher	A	Terrestrial	104	0	237	0
<i>Schoenoplectiella supina</i> (L.)Lye	P	Aquatic	41	1737	0	184
<b>Euphorbiaceae</b>						
<i>Caperonia castaneifolia</i> (L.) A. St.-Hil.	P	Aquatic	34	0	5	0
<i>Croton glandulosus</i> L.	P	Terrestrial	35	0	0	0
<i>C. trinitatis</i> Millsp	P	Terrestrial	104	0	103	0
<i>Euphorbia hirta</i> L.	A	Terrestrial	46	0	29	0
<i>E. thymifolia</i> L.	A	Terrestrial	188	0	146	0
<i>Microstachys hispida</i> (Mart. & Zucc.) Govaerts	P	Terrestrial	27	0	0	0
<b>Heliotropiaceae</b>						
<i>Euploca filiformis</i> (Lehm.) J.I.M.Melo & Semir	A	Terrestrial	532	0	507	0
<b>Isoetaceae</b>						
<i>Isoetes pedersenii</i> H.P. Fuchs ex E.I. Meza & Macluf	P	Amphibious	0	698	0	549
<b>Lamiaceae</b>						
<i>Hyptis brevipes</i> Poit.	A	Terrestrial	804	0	1055	0
<i>H. lorentziana</i> O. Hoffm.	A	Amphibious	58	0	79	0
<b>Leguminosae</b>						
<i>Aeschynomene fluminensis</i> Vell.	P	Amphibious	2	0	0	0
<b>Lythraceae</b>						
<i>Rotala ramosior</i> (L.) Koehne	A	Amphibious	1879	3314	1959	680
<b>Nymphaeaceae</b>						
<i>Nymphaea amazonum</i> Mart. & Zucc.	P	Aquatic	0	21	0	4
<i>N. gardneriana</i> Planch	P	Aquatic	0	533	0	19
<b>Ochnaceae</b>						
<i>Sauvagesia erecta</i> L.	A	Terrestrial	10	0	0	0
<b>Onagraceae</b>						
<i>Ludwigia grandiflora</i> (Michx.) Greuter & Burdet	P	Aquatic	37	0	0	0

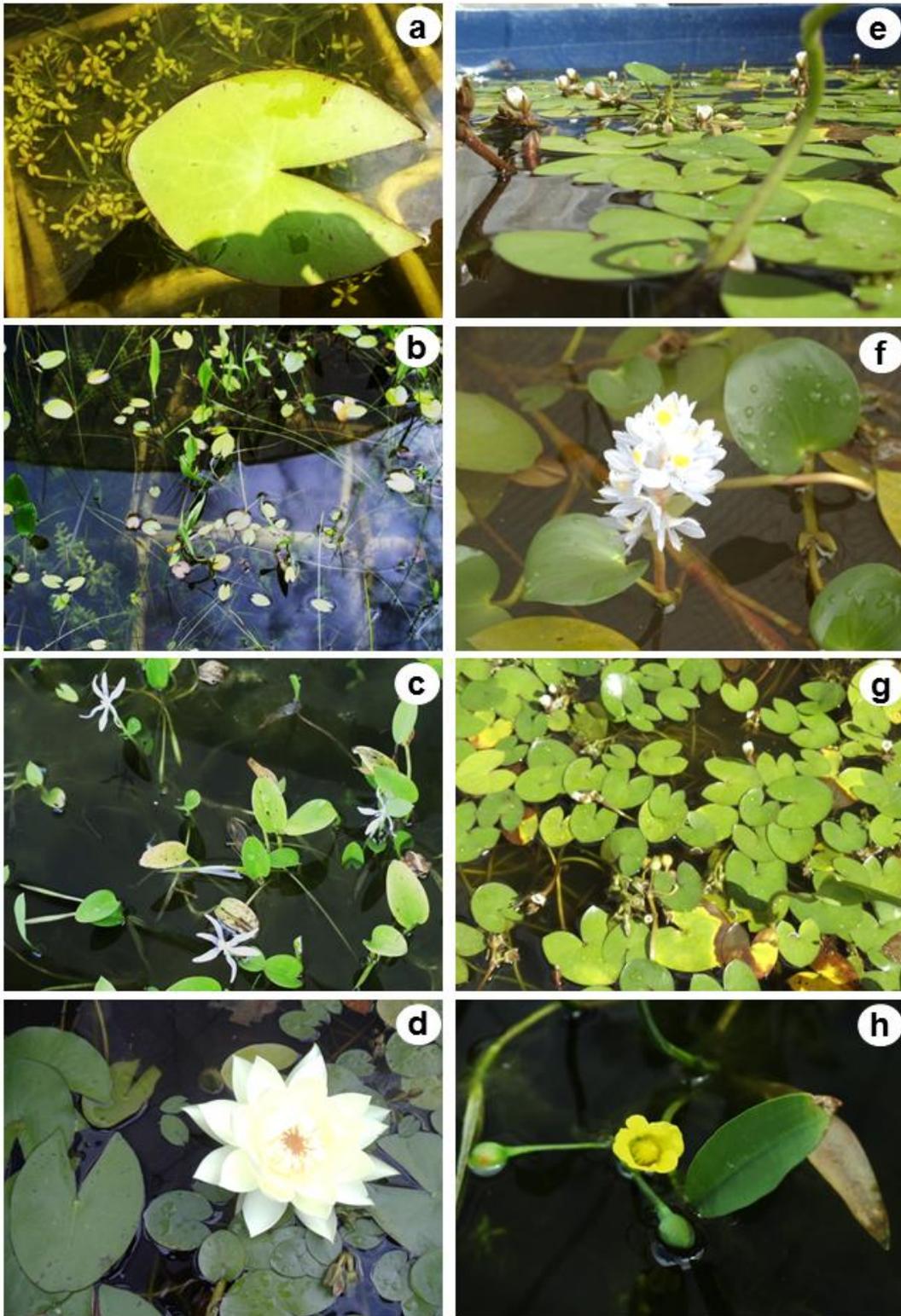
<i>L. inclinata</i> (L. f.) M. Gómez	A	Aquatic	81	33	0	6
<i>L. octovalvis</i> (Jacq.) P.H. Raven	A	Amphibious	1587	0	733	0
<b>Orobanchaceae</b>						
<i>Agalinis glandulosa</i> (G.M. Barroso) V.C. Souza	A	Terrestrial	0	0	15	0
<b>Phyllantaceae</b>						
<i>Phyllanthus orbiculatus</i> Rich	P	Terrestrial	6	0	0	0
<i>P. stipulatus</i> (Raf.) G.L. Webster	A	Amphibious	81	0	19	0
<b>Plantaginaceae</b>						
<i>Angelonia salicariaefolia</i> Humb. & Bonpl.	P	Terrestrial	98	0	28	0
<i>Bacopa australis</i> V.C. Souza	P	Aquatic	470	2208	310	1351
<i>B. myriophylloides</i> (Benth.) Wettst.	P	Aquatic	74	24	0	31
<i>B. salzmännii</i> (Benth.) Wettst. ex Edwall	P	Aquatic	78	87	0	157
<i>B. stricta</i> (Schrad.) Edwall	A	Amphibious	81	0	37	0
<i>Scoparia dulcis</i> L.	A	Terrestrial	5	0	0	0
<i>S. montevidensis</i> (Spreng.) R.E. Fr.	A	Terrestrial	396	0	128	0
<b>Poaceae</b>						
<i>Axonopus purpusii</i> (Mez) Chase	P	Terrestrial	28	0	0	0
<i>Cynodon dactylon</i> (L.) Pers.	P	Terrestrial	2	0	0	0
<i>Digitaria bicornis</i> (Lam.) Roem. & Schult.	P	Terrestrial	121	0	34	0
<i>D. fuscescens</i> (J. Presl) Henrard	P	Terrestrial	13	0	35	0
<i>Eragrostis articulata</i> (Schrank) Nees	A	Terrestrial	10	0	0	0
<i>Hemarthria altissima</i> (Poir.) Stapf & C.E. Hubb.	A	Terrestrial	6	0	2	0
<i>Leersia hexandra</i> Sw.	P	Amphibious	0	21	0	2
<i>Paspalum alnum</i> Chase	P	Terrestrial	23	0	5	0
<i>P. plicatulum</i> Michx.	P	Terrestrial	44	0	0	0
<i>Reimarochloa acuta</i> (Flüggé) Hitchc.	P	Terrestrial	10	0	124	0
<i>Setaria parviflora</i> (Poir.) Kerguélen	A	Terrestrial	274	0	170	0
<b>Pontederiaceae</b>						
<i>Heteranthera limosa</i> (Sw.) Willd.	P	Aquatic	0	231	0	51

<i>Pontederia subovata</i> (Seub.) Lowden	P	Aquatic	0	470	0	102
<b>Portulacaceae</b>						
<i>Portulaca fluvialis</i> D. Legrand	P	Terrestrial	22	0	34	0
<i>P. pilosa</i> L.	A	Terrestrial	10	0	0	0
<b>Rubiaceae</b>						
<i>Borreria eryngioides</i> Cham. & Schltl.	A	Terrestrial	66	0	33	0
<i>Diodia kuntzei</i> K. Schum.	A	Amphibious	145	0	284	0
<i>Richardia grandiflora</i> (Cham. & Schltl.) Steud.	A	Terrestrial	756	0	441	0
<b>Verbenaceae</b>						
<i>Lippia alba</i> (Mill.) N.E. Br. ex Britton & P. Wilson	P	Terrestrial	29	0	0	0

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**Fig S1** Sample-based species accumulation curves of seedlings in flood and post-flood treatment, seasonally flooded grassland in the Pantanal wetland (Central-West Brazil) for (a) dry and (b) flood season. Species accumulation curves are scaled by the number of accumulated individual plants. Points are expected (mean) species richness and transverse lines are confidence intervals.



**Fig S2** Aquatic plants emerged from the seed bank in the flood treatment, (a) seedlings of *Rotala ramosior* and *Bacopa australis*; (b) Submerged trays with some aquatic macrophytes; (c) *Heteranthera limosa*; (d) *Nymphaea gardneriana*; (e) *Sagittaria guayanensis* ;(f) *Pontederia subovata*; (g) *Sagittaria guayanensis* and *Limnocharis flava*; (h) *L. flava*.



**Fig S3** Plants emerged from the seed bank in the post-flood treatment, (a) *R. ramosior*; (b) *Ludwigia octovalvis* and (c) *Helanthis tenellum*, immediately after removal from the flood (d) several herbs germinating 30 days post-flood; (e,f) several seedlings highlighting various grasses 40 days post-flood.

## Capítulo IV

**Seasonal flooding, topography and organic debris  
deposition interact to influence seedling emergence  
and distribution in a seasonally flooded grassland**

Francielli Bao

Tracy Elsey-Quirk, Marco Antonio de Assis, Rafael Arruda and Arnildo Pott

### ABSTRACT

In seasonally flooded wetlands, natural disturbances such as flooding and associated organic debris deposition followed by a drawdown provide opportunities for plant species regeneration, which can promote diversity of the plant community over space and time. Specifically, regeneration of species may be influenced by the direct effect of flooding on seed dispersal and seedling emergence as well as the indirect effect of organic debris on what emergence and recruitment. Our objective was to examine the influence of flooding on seedling distribution by examining the effect of seasonal, topography and cover of organic debris in seasonally flooded grassland of the Pantanal. We measured species richness and abundance of seedlings for three years at three topographic levels with variable coverages of organic debris at the end of the flood season, and during the dry season when there was no debris formation. A total of 43 species were recorded with no difference in species richness between flood stations and dry seasons, suggesting the formation of a persistent seed bank with high potential for regeneration after extreme seasonal events, since the general abundance of species at the end the period was higher. The distribution of seedlings differed between topographic levels after floods, the highest abundance and seedling richness occurred at the medium level, followed by the high and finally the low level. The regeneration potential was observed through annual emergent and perennials of rapid colonization. Our results reinforce that areas with predictable annual floods stimulate individual species adaptation to successful regeneration.

**Keywords:** Neotropical savanna; Seedling regeneration; Wetland.

## INTRODUCTION

Wetlands are naturally disturbance-dependent ecosystems where flood pulsing and drying and, deposition of sediments and organic debris can alter population and community structure and resource and substrate availability (White & Pickett 1985). Disturbances provide opportunities for plant species regeneration and, compared to a static stable system, can promote diversity across temporal and spatial scales (Middleton 1999). In seasonal grassland wetlands, flooding and organic deposition disturbances interact through an increase in aquatic plant cover during the flood period, which subsequently forms extensive mats of organic debris when the water recedes (Marino et al. 2010).

Flooding facilitates dispersal via hydrochory, introducing potentially novel species to an area and redistributing existing propagules (Middleton et al. 2006). Saturated soils and high water will also limit the emergence and survivorship of species not adapted to flooded conditions (Karlin 2016). Because the effects of flooding on regeneration dynamics is highly dependent on elevation, topographic variation across the landscape also influences spatial patterns of plant communities (Zhou et al. 2012). When the flood waters recede, aquatic macrophytes adapted to high water conditions dieback from desiccation. The resulting organic debris can be deposited, indirectly affecting abundances and distributions of species (Wainwright et al. 2017). Organic debris can capture and bury seeds (Dwyer et al. 2015), potentially increasing local seed bank diversity, while reducing sunlight (Ungar & Woodell 1996, Lu et al. 2017), potentially inhibiting the germination and establishment of some species (Abbott & Roundy 2003). Drought conditions during the dry season, may promote a slightly different suite of species depending on species-specific requirements for germination and growth (Casanova & Brock 2000). Thus, periods of flood and drought promote high turnover of species (Garssen et al. 2014, Brotherton & Joyce 2015, Arias et al. 2016, Ma et al. 2017), yet our understanding of disturbance dynamics in seasonally flooded wetlands would be enhanced through a comprehensive examination of how

seasonal flooding, topography, and organic deposition influence regeneration and distribution of seedlings in the seed bank.

Growth form and life history characteristics of species influence their distribution in the seed bank and in the community (van der Valk 1981). For example, during flood periods, the density of submerged and floating macrophytes is high (Brock 2011), as the occurrence of the species, *Hydrocleys parviflora*, *Sagittaria guayanensis*, *Nymphaea amazonum* and *N. gardneriana*. Efficient regeneration after an extreme seasonal event is a determining factor of establishment in grasslands, with the main regeneration mechanism often regrowth of dominant grasses (Lhotsky et al. 2016). However, recruitment from the seed bank can increase the diversity and local genetic variability (Barrett et al. 2005, Baldwin et al. 2010), and assures the regeneration in case the regrowth mechanisms fail (Lhotsky et al. 2016). At the end of the dry period, an increase in soil moisture increases germination of amphibious species and the establishment of the aquatic vegetation (Brock 2011).

The plant community structure of wet grasslands may be highly dependent on local topography where seasonal ponds can form during flooded periods (Pott & Pott 2015), which promote zonation patterns within the vegetation (Damasceno-Junior et al. 2005). One hypothesis is that species abundance and richness are higher in post-flood periods due to greater transport of diaspores (Oliveira et al. 2015, Souza et al. 2016) and, particularly at lower topographies, due to greater retention of seeds (Pagotto et al. 2011, Bao et al. 2014). Seasonal ponds surrounded by flooded grasslands create spatial diversity influenced by the diversity of nearby habitats (Boedeltje et al. 2004, Hopfensperger et al. 2009), and where the cycles of floods and droughts create temporal niches for species adapted to the aquatic and dry environment or to both conditions (Brock 2011).

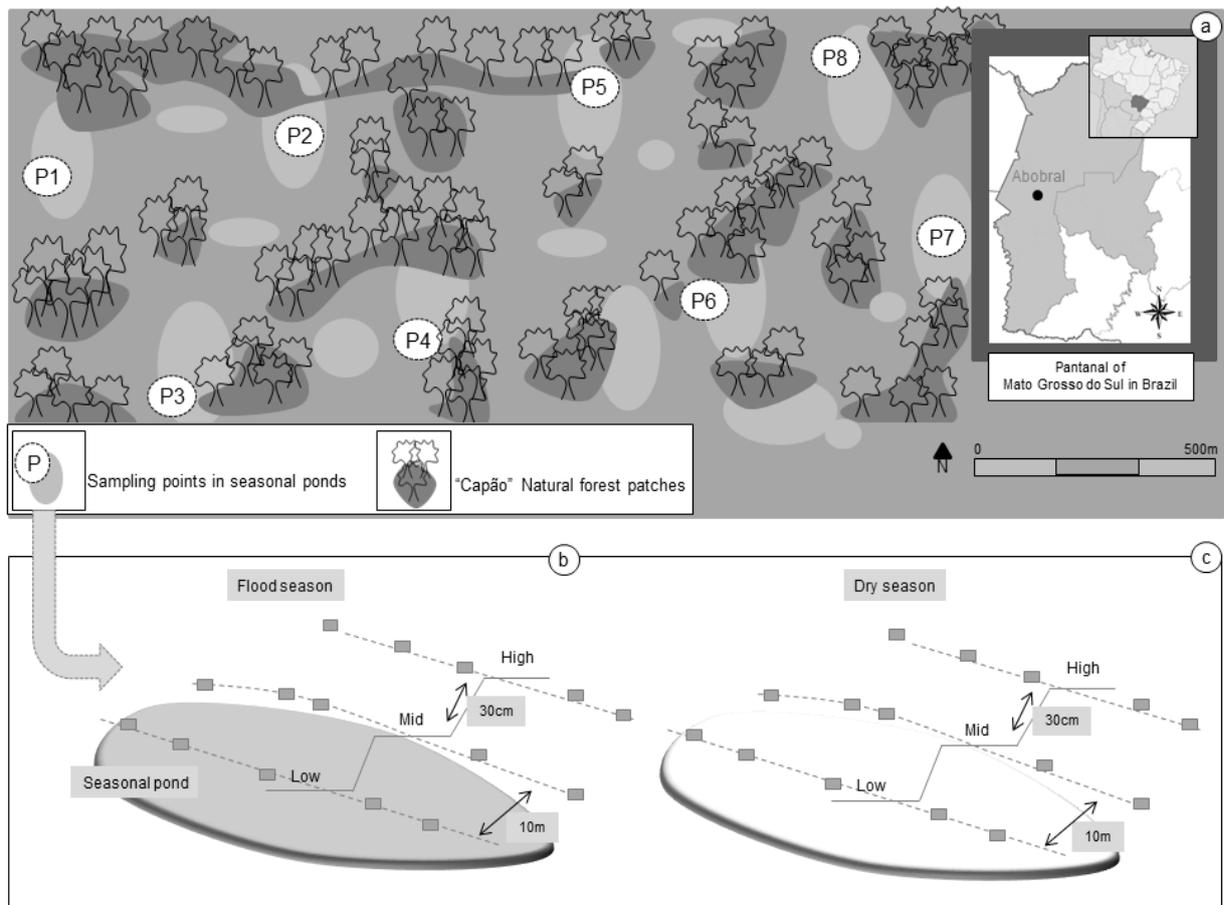
The goal of the present study was to examine how natural disturbances of seasonal flooding and organic debris deposition interact with topography to affected seed bank dynamics in seasonally flooded grassland of the Brazilian Pantanal. Specifically, we tested the hypothesis

seasonal flooding promotes richness and abundance of seedlings through indirect action of deposition of organic debris, except at low topographies where a higher concentration of debris light availability for emergence.

## **METHODS**

### **STUDY AREA**

The study area was a seasonally flooded grassland in the Pantanal wetland (Central-West Brazil, Fig. 1-a). The area is characterized by extensive pastures of exotic and native grasses (Bao et al. 2015), which experience annual floods from local rainfall and river overflow. The flood season typically occurs from April to August and forms extensive seasonal ponds filled with aquatic plants, and a rich seed bank on the floodplain, which is largely influenced by variations in local topography (Bao et al. 2014). The climate of the region is tropical sub-humid, with a mean annual temperature of 26°C (Allem & Valls 1987, Silva & Abdon 1998), and an average annual rainfall of 1100 mm, with maximum (7.34m) and minimum level (2.37m) of the Miranda river (data collected at ranch São Bento, between 2005 and 2015).



**Fig. 1** Seasonally flooded grassland in Pantanal wetland (Central-West Brazil): (a) Sampling design including eight sampling points and representation of the topographic levels (low, mid and high) at each sampling point (b) end of the flood period (formation of seasonal pond, with the accumulation of organic debris) and (c) end of the dry period (dry grassland, absence of organic debris).

#### DATA SAMPLING

Seedlings were sampled in eight seasonal ponds located approximately 1 km apart. Samples were collected during two characteristic hydrophases of the Pantanal wetland (flood and drought), in three years of study: at the end of flood, when the presence of organic debris was observed (between July and August) (Fig.1-b); and after the dry season, when there were no observable organic debris on the grassland (October and November) (Fig. 1-c). Topographic levels were established using a reference of the flood-level water mark on fence poles and plants, considering vertical intervals of ca. 30cm and horizontal distance of ca. 10 m among topographic

levels (i.e., high, mid and low) (cf. Bao et al. 2014, 2015). The percentage (%) of organic debris was estimated visually by the same observer using standardized methods at each topographic level during each sampling period. Points were established in the first year of sampling (2011), and at the end of the flood season, topographic levels were classified into three categories: (i) low – longer duration of flood (waterlogged soil) and largest deposit of organic debris; (ii) mid – intermediate level (wet soil) and (iii) high – short duration of flood (dry soil), with lowest deposition of debris.

In the field, during both flood and dry periods, the number of seedlings was quantified within 0.5 x 0.5 m plots, on each level. At each topographic level, we collected five replicates of seedling counts, placed along a measuring tape from a table of random numbers (e.g., Goodman et al. 2011). Only seedlings that displayed cotyledons up to the second pair of leaves (Brasil 2009) were considered for richness and identification. For seedling identification, field guides (e.g., Kissmann & Groth 1992, 1995, 1997), and assistance from specialists on plants of the Pantanal were used. Seeds of some species were collected germinated in the greenhouse to examine seedling characteristics for comparison to seedlings in the field.

#### DATA ANALYSIS

To determine if species richness will be greater after the flood than after the dry season, we constructed species accumulation curves to evaluate the effect of seasonality and to verify the variation between the topographic levels. Species richness was the accumulated sum of the number of species recorded in the sampling plots. For abundance data, we used the mean number of individuals of each species in the three-seasonal flood-dry cycles and calculated the total sum of abundance per sampling plot. With richness and abundance values as response variables, we constructed a Two-Way ANOVA model with season and topography as predictors. In this model we applied a standardize on response data to zero mean and unit variance. To test whether the cover of organic debris influenced the distribution of seedlings between the topographic levels,

we tested the correlation between the mean percentage cover of organic debris (external variable in the statistical model) with richness and abundance data as response variables only in the dry period. For this analysis, we used ordinary least squares regression model (OLS), which assumes normally distributed errors in the residuals. We compare models without applied a transformation and after standardizing the response variable to zero mean and unit variance and predictor variable by arcsine transformation. These set of models produced similar results, anyway we stay with model without any kind of transformation. To test the influence of percentage of organic debris at each topographic level, we constructed separate OLS models for each level. With richness and abundance data as response variables only in the dry period, we used a one-way ANOVA model where the fixed factor was the topographic level. In this model we applied a standardize on response data to zero mean and unit variance. If necessary, we used Tukey's test for post-hoc comparisons in all ANOVA models. All analyses were conducted in R environment (R Core Team 2017), with biodiversity R (Kindt & Coe 2005), vegan (Oksanen 2015), lattice (Deepayan 2008) and permute (Simpson 2016).

## RESULTS

### RICHNESS AND ABUNDANCE OF THE SEEDLINGS

A total of 43 species representing 19 families and 37 genera were found in the grassland over a three year sampling period. Thirty-six species of seedling occurred following the flood season (56% annuals; 44% perennials) and 31 species in the dry season (65% annuals; 35% perennials) (Table S1). Poaceae and Cyperaceae had the greatest number of species (five species each), followed by Euphorbiaceae and Rubiaceae (four species each).

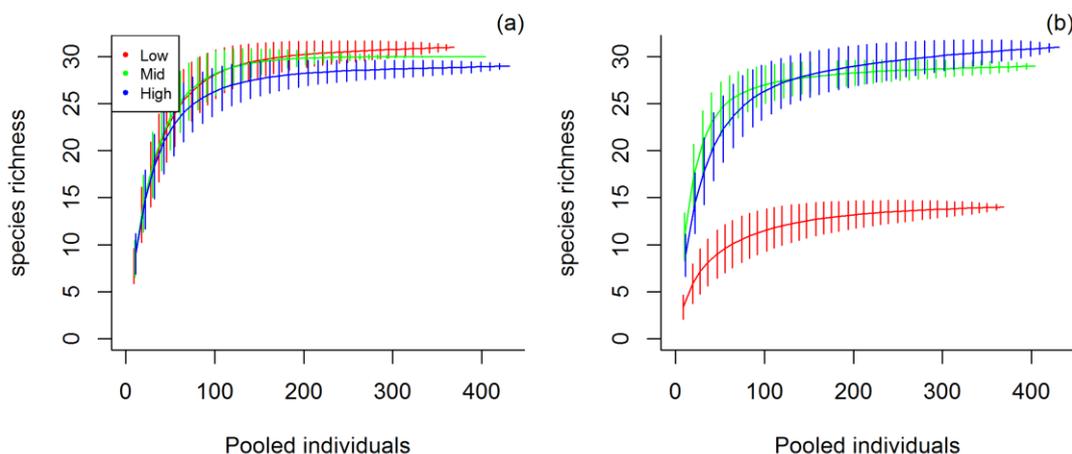
Species richness did not significantly differ between flood and dry seasons (Table 1). However, six aquatic macrophyte species found only at the end of the flood period were *Bacopa australis*, *Cyperus surinamensis*, *Helanthisium tenellum*, *Ludwigia inclinata*, *Pontederia subovata*

and *Schoenoplectiella supina*. Four species exclusive to the dry period were *Axonopus purpusii*, *Euploca filiformis*, *Lippia alba* and *Portulaca pilosa* (Table S1).

**Table 1** Analysis of variance of the abundance and average seedling richness between the seasons and topography, in seasonally flooded grasslands in Pantanal wetland (Central West Brazil).

	Response variable	Source of variation	Df	F value	P value
Two-Way ANOVA	Seedling richness	Season	1	0.39	0.532
		Topography	2	95.59	<0.001
		Season*Topography	2	62.65	<0.001
		Residuals	234		
	Seedling abundance	Season	1	269.49	<0.001
		Topography	2	92.61	<0.001
		Season*Topography	2	80.58	<0.001
		Residuals	234		
One-Way ANOVA	Seedling richness (only dry season)	Topography	2	3.70	0.028
		Residuals	117		
	Seedling abundance (only dry season)	Topography	2	13.94	<0.001
		Residuals	117		

The accumulation curves illustrate that most species of the studied community were sampled. Season interacted with topographic level to influence species richness (Table 1, Fig. 2). In general, the number of species that emerged from the mid- and high topographic levels post-flood was similar to that found at all topographic levels during the dry season (Fig. 2a). Species richness vary among topographic levels in dry season and, in flood season species richness was significantly lower at the low topographic level than at the mid- and high levels (Table 1, Fig. 2b).



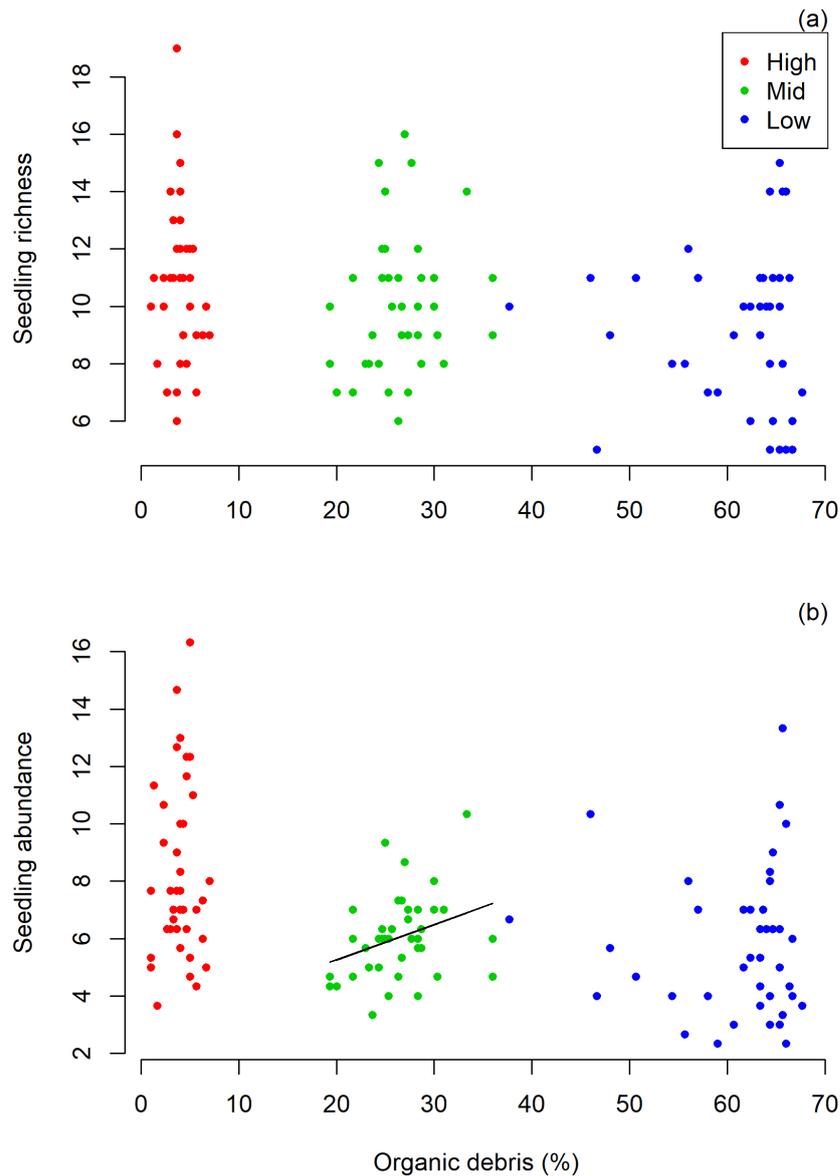
**Fig. 2** Sample-based species accumulation curves of seedlings in a seasonally flooded grassland in the Pantanal (Central-West Brazil) at three topographic levels (low, mid and high) in the (a) dry season and (b) flood season.

The abundance of seedlings at the end of the flood was three times greater than that following the dry season (Table 1). The most abundant species of seedlings were *Richardia grandiflora* (975 seedlings) and *Rotala ramosior* (991) at the end of the flood season, and *Diodia kuntzei* (129) and *R. grandiflora* (230) at the end of the dry season (Table S1). Seedling abundance was significantly greater at the high-level than at low- and mid- topographies across seasons (Table 1). All topographic levels had a similar suite of species, of which the most abundant were *Diodia kuntzei*, *Hyptis brevipes*, *Ludwigia octovalvis*, *Microstachys hispida*, *Richardia grandiflora* and *Staelia thymoides*.

#### ORGANIC DEBRIS AND TOPOGRAPHY vs PLANT SPECIES DISTRIBUTION

At the end of the flood season, the percent cover of organic debris ranged from 10 to 70% across topographic levels. The low-level presented the highest deposition of debris (up to 70%), varying in mean ( $63 \pm 0.099$ ), already mid-level (up to 50%), with a mean variation ( $38 \pm 0.068$ ) and finally the high-level had the lowest deposition (up to 10%) with mean variation of ( $7 \pm 0.033$ ). Species richness was not significantly related to coverage of organic debris (Fig. 3-

a). Within topographic levels, organic debris had a positive effect on seedling abundance at mid-level (Adjusted  $r^2 = 0.08$ ,  $P=0.04$ , Fig. 3-b). Additionally, the environmental conditions found in the mid-level favored the occurrence of aquatic, amphibious and terrestrial species, such as *Bacopa australis*, *L. octovalvis*, *R. grandiflora*, *Rotala ramosior* and *Scoparia montevidensis* (Table S1).



**Fig. 3** Relationships of (a) species richness and (b) seedling abundance with organic debris (%), on three topographic levels in floodable grasslands: low, mid and high in seasonally flooded grasslands, Pantanal wetland (Central West Brazil). We built separate ordinary least squares regression model (OLS) for each topographic level.

Low topographic levels had low abundances and few species at the end of the flood season (Fig. 3, Table S1). Most species were aquatic, e.g. *Eleocharis minima*, *L. inclinata*, *P. subovata* and *Schoenoplectiella supina*. On the high-level where there was less cover of organic debris and the soil was more well-drained and terrestrial species such as *Croton trinitatis*, *D. kuntzei*, *H. brevipes* and *R. grandiflora*, and grasses, *Axonopus purpusii*, *Reimarochloa acuta* and *Setaria parviflora* had the highest abundances (Table S1). In the dry season there was no presence of organic debris and the distribution of seedlings among topographic levels was homogenized (Table 1).

## DISCUSSION

### SEASONALITY OF SEEDLING RICHNESS AND ABUNDANCE

The number of species that emerged as seedlings was similar between flood and dry seasons. Although there was not a significant difference between the seasons, the flood season, however, presented some species of exclusive macrophytes of the period reflecting the importance of floods, mainly in terms of abundance of the seed banks (Hopfensperger et al. 2009, Oliveira et al. 2015, Pagotto et al. 2011), which reflects on the abundance of the seedlings bank. This reinforced our hypothesis that the seasonal floods promote the wealth and abundance of the community. The occurrence of the same species at both end of flood and end of drought suggest the formation of a persistent seed bank (Brock 2011), rich in annual plants that tend to appear subsequently to an environmental change (Capon 2007).

During periods of dry conditions and severe droughts seedlings adapted to flood events do not survive to become a part of the adult plant community (Bunker & Carson 2005, Lopez & Kursar 2003). However, the presence of aquatic plants in the both flood and dry seasons is a consequence of their life form (Capon & Brock 2006), phenotypical plasticity (Scremin-Dias et al. 2011) and evolutionary adaptation, allowing them to establish in various habitats and moisture conditions (Jackson & Colmer 2005). Thus, flooding promotes germination of some

species not found in dry, attributed to the flood periodicity and environmental restrictions (Rahel 2000). While richness did not differ between seasons, seedling abundance was three times greater at the end of the flood, when water drawdown can enhance germination. Several studies have demonstrated an increase in germination from the seed bank in post-flood periods (Baldwin et al. 2001, Hölzel & Otte 2004, Baskin & Baskin 2014, Souza et al. 2016). The highest seedling abundance was found in *D. kuntzei*, *H. brevipes*, *L. octovalvis*, *R. grandiflora* and *Rotala ramosior* in both periods occurs because they are pioneer species in dried out ponds and seasonal streams (Pott & Pott 1994), and are very abundant in the seed bank in the region (Bao et al. 2014, Souza et al. 2016).

Seedling abundance differed among topographic levels in the post-flood period, where high-level had the highest abundance. The homogenization in seedling distribution among the topographic levels in the dry period is due to the uniform environmental conditions, such as the absence of shading by organic debris and adequate soil moisture, fundamental factors for seed germination and seedling recruitment (Kozłowski & Pallardy 2002). When seeds are subjected to changes in environmental conditions, some are adapted to such changes and germinate, while others remain dormant until conditions become suitable (Dudley & Schmitt 1996), promoting variation in the composition and distribution of seedlings, as seen after the flood. Hydrochory during the flood period (Hampe 2004) can ultimately promote a homogeneous seed bank in the dry period (Johanson et al. 1996).

#### ORGANIC DEBRIS AND TOPOGRAPHY vs PLANT SPECIES DISTRIBUTION

Although the composition of plant species was favored by flooding, abundance of seedlings varied according to the interaction between topography and the amount of organic debris. Thus, seedling recruitment was favored by the protection from the organic debris. The debris formed a sort of protective covering, which was later lifted by grass regrowth, until it was disrupted by rains or grazing.

In the Pantanal, organic debris deposition occurs seasonally, only being observed in the post-flood season. Topography plays an important role distribution pattern of organic debris depending on flooding dynamics (Kellogg et al. 2003). Organic debris coverage was greatest at the low topographic level where species abundance of seedlings was it was higher at high level. The variation in topography promotes differently seed capture according to the lowering of water (Middleton & Mckee 2011), as well as zonation patterns of species (Cattanio et al. 2002) such as the colonization of terrestrial plants at high topographies, and aquatic species at low topographies (Bao et al. 2015). In low areas, we observed changes in seedling abundance according to the accumulation of organic debris, where levels with higher amounts of organic debris, the lower abundance and richness of seedlings.

Generally, seed germination increases with increasing soil moisture in flooded areas when compared to non-inundated soil (Capon & Brock 2006). However, the high build-up of organic debris in certain places may inhibit seed germination of some species (Facelli & Pickett 1991). Many of these species may have seeds or seedlings sensitive to the effects of flood limiting their distribution (Scarano 1998). Furthermore, seeds of species not adapted to anaerobic conditions may be eliminated by long periods of inundation (Poiani & Dixon 1995). We hypothesize that lower richness and abundance of seedlings on the low-level was associated with the duration of the flood and soil waterlogging as well as greater deposition of organic debris, which decreased incident light. Nonetheless, we can also raise the question that in low-levels there is high density of seeds (Middleton 2002), in such case, the belowground competition can be a factor influencing selection of species during germination.

Light limitation is the main factor reducing diversity under large quantities of accumulated dead matter (Carson & Barrett 1988). In contrast, the mid and high-levels were characterized by fast germination of terrestrial and amphibious species, with the terrestrial species exhibiting rapid colonization following water drawdown (Skoglund & Hytterborn 1990). The higher abundance and richness on the mid-level can be the result of the fast post-flood

colonization of annual and short lived species. The plasticity of some species to germinate and establish in flooded or dry areas is an important factor in regeneration of wetlands (Johanson et al. 1996, Hampe 2004). Each species requires its optimal conditions for germination, generally dependent on quantity of light and water (Marod et al. 2004, Ruger et al. 2009).

Flooding in the Pantanal is considered an environmental filter that acts on the regulation of richness and abundance of the seed bank and of the grassland species (Bao et al. 2014, 2015). The study of the recruitment of seedlings immediately after extreme seasonal events provided us with information on the knowledge of which species present a seed bank capable of recolonization. In summary, the regeneration via soil seed bank occurred by fast colonization emergent species, e.g., *Diodia kuntzei*, *Ludwigia octovalvis*, *Praxelis clematidea*, *Richardia grandiflora*, *Rotala ramosior*, observed in both seasons. We highlight that may have been some sampling bias, since samples were taken soon after flood receded and begin of rains, and some late species could have been missed in the list of seedlings, though not seen in the vegetation (cf. Bao et al. 2015) neither in the seed bank (cf. Bao et al. 2014). For example, grasslands in the Pantanal show high richness of grasses (ca. 24 species) in the vegetation cover (cf. Bao et al. 2015), however among the seedlings we recorded only five species (Table 1). Most Neotropical grasses present dormancy as key mechanism to avoid germination during environmental instability (Ramos et al. 2017). Although resprouting was not evaluated, this mechanism was visible in the field, and probably is the main source of reestablishment of such species after an extreme seasonal event, as occurs in other grasslands (Klimešová & Klimeš 2007, Ott & Hartnett 2011, Deng et al. 2013, Herben et al. 2016). More studies should be done on grasslands involving resprouting mechanisms after extreme seasonal events, as well as evaluation of survival of grasses submitted to flooding experiments.

In this wet zone we observed the importance of flooding in the composition and distribution of the seedlings bank, where the seasonal inundation regime contributed to increase the production of organic debris, which can act as traps to capture seeds (Neff & Baldwin 2005)

and thereby increase local abundance, beside modifying the distribution of seedlings according to the germination requirements of each species. Our results indicate that flood reflects in the grassland regeneration potential and is a strong selective force driving the individual strategies of the species for successful.

#### ACKNOWLEDGEMENTS

The authors thank to INAU (Instituto Nacional de Ciência e Tecnologia de Áreas Úmidas, or Brazilian Institute for Wetlands), for support and finance of the project, and to the Brazilian governmental agencies CNPq for the scholarship (F.B.) and research grants (A.P. and M.A.A.) and CAPES for Visiting Professor grant (A.P.).

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## **Supplementary Material**

**Table S1** List of species present (abundance) in the grassland, classified about the life cycle, distributed between levels (L, low; M, mid; H, high) under seasonal variation (flood and dry seasons) in the Abobral sub-region of the Pantanal wetland (Central West Brazil).

Family/species	Life Cycle	dry season			flood season		
		L	M	H	L	M	H
<b>Alismataceae</b>							
<i>Helanthium tenellum</i> (Martius) Britton	P	-	-	-	40	81	-
<b>Asteraceae</b>							
<i>Praxelis clematidea</i> (Griseb.) R.M.King & H.Rob.	A	11	20	18	-	76	48
<i>Sphagneticola brachycarpa</i> (Baker) Pruski	P	15	12	18	6	160	62
<i>Stilpnopappus pantanalensis</i> H.Rob.	A	33	32	38	-	59	42
<b>Cyperaceae</b>							
<i>Cyperus haspan</i> L.	P	-	-	-	11	88	15
<i>C. surinamensis</i> Rottb.	A	-	-	-	2	47	33
<i>Eleocharis minima</i> Kunth	A	12	12	-	35	82	-
<i>Kyllinga brevifolia</i> Rottb.	P	6	19	15	1	22	18
<i>Schoenoplectiella supina</i> (L.) Lye	P	-	-	-	47	34	-
<b>Euphorbiaceae</b>							
<i>Caperonia castaneifolia</i> (L.) A.St.-Hil.	P	8	18	7	-	-	11
<i>Croton trinitatis</i> Millsp.	A	33	42	55	27	138	120
<i>Euphorbia thymifolia</i> Burm.	A	29	43	60	-	37	48
<i>Microstachys hispida</i> (Mart.) Govaerts	P	36	29	84	-	39	49
<b>Heliotropiaceae</b>							
<i>Euploca filiformis</i> (Lehm.) J.I.M.Melo & Semir	A	7	23	28	-	-	-
<b>Lamiaceae</b>							
<i>Hyptis brevipes</i> Poit.	A	23	37	76	-	275	170
<i>Hyptis lorentziana</i> O. Hoffm.	A	9	12	11	13	57	25
<b>Lythraceae</b>							
<i>Rotala ramosior</i> (L.) Koehne	A	29	54	38	235	499	257
<b>Malvaceae</b>							
<i>Melochia arenosa</i> Benth	P	-	-	-	-	-	14
<i>M. simplex</i> A.St.-Hil.	P	11	26	43	-	-	22
<i>Sida cerradoensis</i> Krapov.	P	7	12	6	-	-	20
<b>Onagraceae</b>							
<i>Ludwigia grandiflora</i> (Michx.) Greuter & Burdet	A	2	4	1	7	22	-
<i>L. inclinata</i> (L. f.) M. Gómez	A	-	-	-	5	38	2
<i>L. octovalvis</i> (Jacq.) P.H. Raven	A	57	33	70	117	361	210
<b>Phyllantaceae</b>							
<i>Phyllanthus stipulatus</i> (Raf.) G.L. Webster	A	13	7	15	-	28	20
<b>Plantaginaceae</b>							
<i>Angelonia salicariifolia</i> Bonpl.	P	19	26	35	-	75	27
<i>Bacopa australis</i> V.C.Souza	A	-	-	-	206	192	84
<i>Scoparia montevidensis</i> (Spreng.) R.E. Fr.	A	37	40	41	-	253	157
<b>Poaceae</b>							
<i>Axonopus purpusii</i> (Mez) Chase	P	5	6	9	-	-	-
<i>Digitaria fuscescens</i> (J. Presl) Henrard	P	2	-	-	-	-	42

<i>Eragrostis articulata</i> (Schrank) Nees	A	7	15	37	-	-	-
<i>Reimarochloa acuta</i> (Flüggé) Hitchc.	P	-	-	-	-	9	56
<i>Setaria parviflora</i> (Poir.) Kerguélen	A	23	31	31	-	-	5
<b>Polygalaceae</b>							
<i>Polygala molluginifolia</i> A. St.-Hil. & Moq.	A	38	26	30	-	-	4
<b>Pontederiaceae</b>							
<i>Pontederia subovata</i> (Seub.) Lowden	P	-	-	-	14	3	-
<b>Portulacaceae</b>							
<i>Portulaca pilosa</i> L.	A	16	7	12	-	-	-
<b>Rubiaceae</b>							
<i>Borreria eryngioides</i> Cham. & Schltldl.	A	15	8	25	-	8	1
<i>Diodia kuntzei</i> K. Schum.	P	55	29	45	-	164	239
<i>Richardia grandiflora</i> (Cham. & Schltldl.) Steud.	A	70	61	99	-	471	504
<i>Staelia thymoides</i> Cham & Schltldl.	A	33	18	22	-	62	108
<b>Verbenaceae</b>							
<i>Lippia alba</i> (Mill.) N.E. Br. ex Britton & P. Wilson	P	31	23	35	-	-	-

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## **Capítulo V**

# **Maintenance of the plant community in the wetlands: efficient role of the seed bank in the diversity of native plants**

Francielli Bao

Tracy Elsey-Quirk, Marco Antonio de Assis, Arnildo Pott

### Abstract

Wetlands dominated by grasses often have introduced exotic grasses, which are able to compete with native plants due to their high tolerance to flood and drought events. Flooding can also facilitate seed dispersal of exotic species, which, following successful germination and establishment, can reduce the diversity of native species. To evaluate the whether seed banks maintain a diverse native plant community in a wetland grassland where exotic plant species dominate the standing vegetation, we compared seed bank and vegetation composition of two areas of grassland, one comprised of native plants and another dominated by the exotic grass, *Urochloa humidicola*, in the Brazilian Pantanal. We recorded a total of 138 species in the grassland areas, with a predominance of annual plants in both the seed bank and vegetation. In both grassland, the bank and the vegetation were distinct, the seed bank showed a greater diversity of species, independent of the dominance of *U. humidicola* in the vegetation. The bank of native and cultivated grassland was significantly distinct due to the positive correlation of aquatic plants in native grassland (91.08 seeds mean), and negative in the cultivated (52.96 seeds). Yet in the vegetation, amphibious plants predominated in the grassland, correlating positively the native and cultivated grassland. The bank presented potential to maintain the diversity of native species in this community, since the flood and dry cycle promoted the presence of distinct species into the seed bank, but the lower richness in the vegetation reflects the dependence on the germination stage. The seasonality floods and droughts influence distinct growth forms, increasing the diversity of both the bank and vegetation. Our results indicate that the presence of *U. humidicola* should be treated with care so as to not reduce the bank of macrophytes in cultivated grassland.

**Keywords:** exotic plants, flood dynamics, grassland, growth forms, seasonal savanna

## Introduction

Wetland grasslands are composed of a diversity of perennial grasses and annual herbs, which vary in abundance according to cycles of flood and drought (Touzard et al. 2002). The Brazilian Pantanal is dominated by open grasslands under periodical floods from local rains and river overflow (Pott and Silva 2015). The seasonality of rain in these wetlands is a determining factor in establishment of the vegetation, due to the direct effects of soil moisture on germination and establishment of seedlings (Baskin and Baskin 2014). The variations in water regime can change over seasons and produce seasonal communities of distinct plants (Casanova and Brock 2000, Harwell and Havens 2003). During the flood season, the vegetation is structured by submerged and floating aquatic macrophytes (Pott and Pott 2004), and as the water recedes, the grasslands become drained and the vegetation is rapidly dominated by emergent species (Pott and Silva 2015).

Grasses have high representation in these grasslands and coexist with the other groups of plants over the seasons (Ma et al. 2012). The predominance of grasses, however, makes these areas ideal for pastoral activities. The search for species resistant and able to compete with aquatic plants during floods leads to the introduction of exotic grasses in natural grasslands worldwide (Bossuyt et al. 2006, Fisher et al. 2009). In the Pantanal, the introduction of *Urochloa humidicola*, utilized as pasture for cattle, became recurrent due to its ability to tolerate flooding (Mattos et al. 2005, Souza et al. 2006) and high regrowth and vegetative propagation potential (Michelan et al. 2010). The morphology of its diaspores (with palea and glume well coupled) provides slow absorption of water allowing it to stay dormant in the field for long periods of time (Montorio et al. 1997). Flooding can prompt a break in dormancy, allowing germination, (LaDeua and Ellison 1999, Baskin and Baskin 2014), in addition to facilitating the dispersal of seeds. Widespread dispersal of *U. humidicola*, can lead to increased dominance in the community at the expense of native species both in the vegetation and in the seed bank (Yang and Li 2013).

The dispersal of seeds and the formation of a bank dominated by exotic species is a great concern in wetlands, as connected waterways can act as corridors increasing the spread of the species through hydrochory (Nathan and Muller-Landau 2000), ultimately modifying local and potentially larger scale native plant community patterns (Whittaker et al. 2001). Wetlands and hydrologic connectivity within and among wetland areas can facilitate changes in the composition and diversity of the vegetation by providing dispersal corridors (Middleton 2002), and modify the composition of the local bank (Boedeltje et al. 2002, Baldwin et al. 2010).

The study of the seed bank in grasslands generates interest in basic and applied ecology, due to the economical need to understand the potential of the bank in regeneration for maintenance of species diversity (Rice 1989). Wetlands of high diversity contain multiple genotypes, promoted by repeated natural disturbances due to the exposure to annual floods (Baldwin et al. 2010), so seeds of high viability and genetically diversity are produced that ensures efficient regeneration (Westcott et al. 1996). Maintenance of species diversity by emergence and regeneration of species from seed banks can be a determinant factor for the establishment and diversity of many plant communities (e.g., van der Valk and Davis 1978, 1979, van der Valk 1981, Bonis et al. 1995, Brock and Rogers 1998, Boedltje et al. 2002, Bernhardt et al. 2008, Brock 2011). Success of regeneration from seed banks, and its influence on species richness and distribution patterns depend on both species-specific germination and growth strategies and environmental conditions (Baider et al. 1999, Peterson and Baldwin 2004).

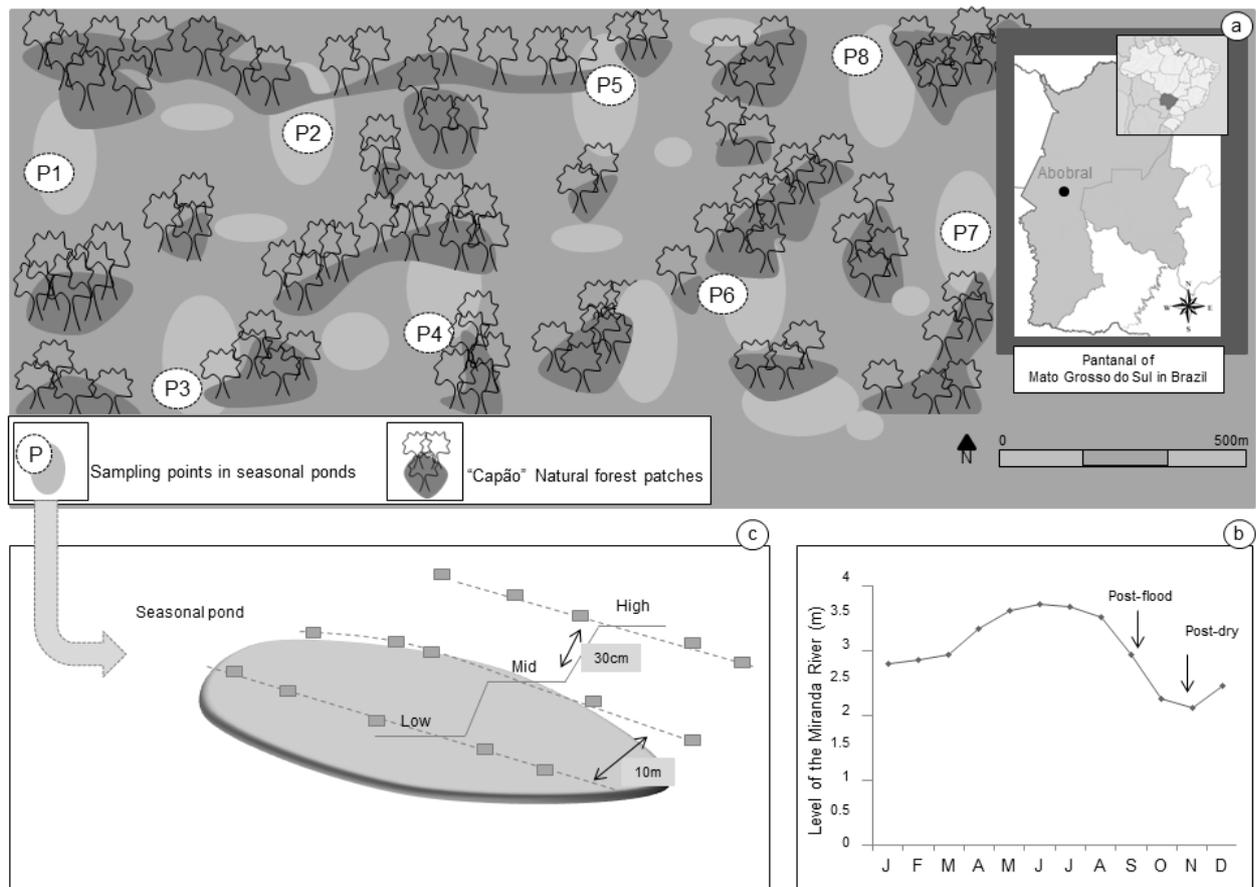
Despite several studies on the role of seed banks in wetlands (e.g., Brock 2011, Goodman et al. 2011, Ma et al. 2012, Beas et al. 2013, Yang and Li 2013, Elsey-Quirk and Leck 2015), few have specifically examined the influence of exotic plant species on the composition of the seed bank and vegetation. Yet, it is necessary to consider how plant species invasion alters plant community and seed bank structure for future protocols of conservation and preservation of wetlands, motivating the need for studies that compare the composition of the aboveground vegetation with the seed reserves in the soil.

For this study, we compared the vegetation and seed bank of seasonally flooded grassland dominated by the exotic *U. humidicola* and another composed of native grasses. This research was guided by the following hypotheses: (i) the presence of the dominant specie *U. humidicola* in the vegetation reduces the similarity of standing vegetation and seed bank; (ii) seasonal flooding played a large role in the entry of seeds by increasing the richness and abundance of native plants in the seed bank; and (iii) nevertheless, the seed bank of *U. humidicola* areas contains some native species diversity, if the released from competition with *U. humidicola* at the establishment stage.

## **Methods**

### **Study area**

The present study was carried out in the sub-region of Abobral, Pantanal wetland, Mato Grosso do Sul (Central-West Brazil, Fig 1a). The climate of the region is tropical sub-humid, with a mean annual temperature of 26°C and an average annual rainfall of 1100 mm (Allem and Valls 1987, Silva and Abdon 1998). The Brazilian Pantanal is regulated by the flood pulse, the seasonality in this region is marked by well defined periods of rain/flood and drought, characterized by annual flood (between February and May), with pluvial and fluvial fluctuations, with maximum (7.34m) and minimum level (2.37m) of the Miranda river (data collected at ranch São Bento, between 2005 and 2015, Fig 1b). The ground level can vary between 1 to 60 cm (Allem and Valls 1987, Silva and Abdon 1998), such variation of topography added to the different sites of hydrological regime form permanent and periodically flooded zones (Pott and Silva 2015).



**Fig 1** Seasonally flooded grassland in the Pantanal wetland (Central-West Brazil). (a) Sampling points including eight seasonal ponds (P1, P2, P3 and P4 in grassland dominated by *U. humidicola*, and P5, P6, P7 and P8 in native grassland), (b) representation of the transects following the topographic levels (low, mid and high) in each sampled pond, with five random samples, (c) mean monthly level of the Miranda River, arrows showing the sampled seasonal periods (post-dry and post-flood), between the years 2005-2015).

To examine the effect of seasonality on the grassland over time, we conducted four samplings on the hydrophases (flood and drought) of the Pantanal: two at the end of the dry period (September) and two at the end of the flood period (July) (Fig 1b). These native grasslands are traditionally utilized as pastures and were partially replaced by cultivated grassland using the exotic *Urochloa humidicola* (Rendle) Morrone & Zuloaga (Poaceae). This species was first introduced 15 years ago.

Samplings were made in eight seasonal ponds, 1 km apart: four located in grassland dominated by the exotic *U. humidicola* and another four in native grassland. To achieve the widest amplitude in capture of seeds and species richness, in each seasonal pond we set three transects according to the flood level: one placed in the middle of the pond (low), one on the edge (mid) and another most external to the pond (high), keeping a distance between them ca. 10m horizontally and ca. 30cm vertically (Fig 1c). The transects were established using as reference the water mark of the flood level on fence poles and taller plants, and the points were marked in the first year of the study, at the end of flooding, when seasonal ponds are formed (cf. Bao et al. 2014, Bao et al. 2015).

#### Vegetation and seed bank sampling

For analysis of the established vegetation, we performed sampling of plant cover using the method of percentage of species present in the study area (e.g., Dolle and Schmidt 2009). In each transect we sampled five random replicates for evaluation of plant cover, totaling 15 samples per pond. Within a delimited area of 0.50 x 0.50m, we made the visual estimate of the percentage cover of each species, and for more precise estimates each quadrat was subdivided with strings into quarters and the observation was always made by the same person.

In each sampling quadrat of plant cover we took three soil samples, each with 20x20cm and 3cm deep, (cf. Bao et al. 2014). Each soil sample was submitted to a distinct method of evaluation of the seed bank – seedling emergence in the greenhouse - the soil samples were spread in plastic trays (30cm x 30cm x 10cm), over a 2 cm layer of sterilized washed sand under ambient temperature, the seedlings of germinated seeds were counted, identified and removed, to avoid competition from new seedlings (Thompson et al. 1997), this method was applied for determining the number of viable seeds in the soil by the manual counting of seeds. For this, soil was washed through a set of sieves with three mesh sizes (0.25mm, 0.35mm and 0.50mm) to trap seeds of distinct sizes (Bonis et al. 1995, Mcfarland and Shafer 2011), to determine the total

number of seeds in the sediment (Simpson et al. 1989). The retained seeds were fixed in alcohol 70% and preserved. In the laboratory the seeds were counted and identified under stereoscopic microscope. Seedling emergence was determined by spreading soil samples in plastic trays (30cm x 30cm x 10cm) and then subjected samples to a flooding regime to simulate a three month flood. Samples were submerged in tanks under 90 cm of water (flood) for three months (filamentous algae was removed when necessary).

The identification of the species was done by comparison with specimens in the Herbarium CGMS, of Universidade Federal de Mato Grosso do Sul (UFMS), consulting specialized books (e.g., Pott and Pott 1994, Pott and Pott 2000, Kissmann and Groth 1992, 1995, 1997) and identification manuals (e.g., Gil and Bove 2006, Groth 1983, Kaul 1978, 1985, Souza and Giuliatti 2014), and collaboration from specialists on plants of the Pantanal. Species were presented according to APG IV- Angiosperm Phylogeny Group (2016). Species germinated from the seed bank were classified according to their life cycle (perennial or annual) and growth form (aquatic, amphibious and terrestrial) (Pott and Pott 1994, Leck and Brock 2000, Pott and Pott 2000).

#### Statistical analysis

First, a descriptive evaluation of the plant community under study was made from data of richness, abundance of the seed bank and cover percentual da vegetation of annual and perennial plants, in the periods of flood and drought, and analysis of variance (ANOVA) was conducted for each grassland type. The similarity between sampled sites according to the relative abundance of the soil seed bank and relative cover of the vegetation (data of grassland and seasonality) was determined using the percentage of similarity (SIMPER). Species diversity of the vegetation and seed bank was calculated using the Shannon diversity index to evaluate the effect of seasonality on diversity in each grassland type, the analysis of variance (ANOVA) and the Tukey test were applied to verify significant mean differences.

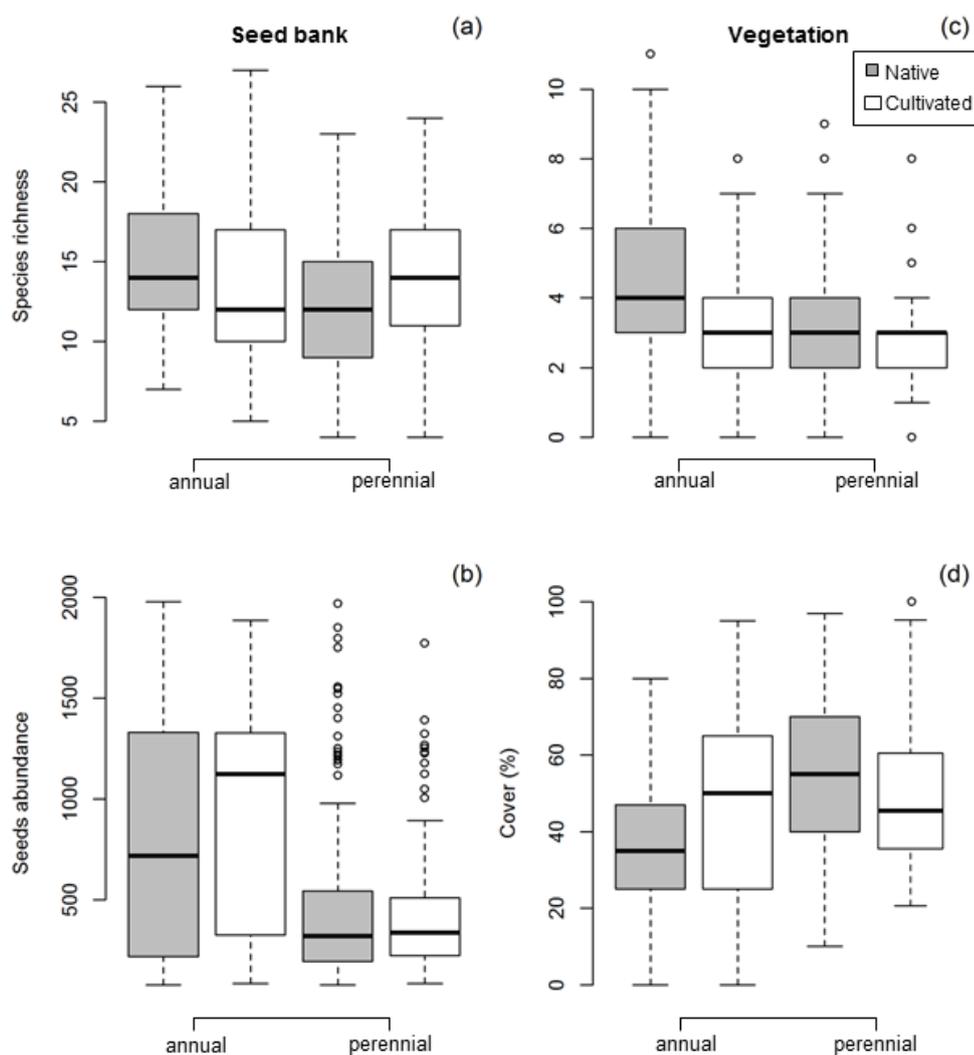
Furthermore, the data of abundance and relative cover of the species in each grassland type and seasonal period were coordinated by Non-Metric Multidimensional Scaling (NMDS), utilizing the Bray-Curtis distance. PERMANOVA was applied to determine if significant mean differences occurred in a set of data by means of Pillai-Trace statistics. We applied analyses of redundancy (RDA) to examine if the growth forms (aquatic, amphibipus and terrestrial) are related to the distribution of species composition of the seed bank and of the vegetation between native and cultivated grassland. To select the growth forms for the analysis we considered the germination and ecological requirements of the plant species to become established during the flood and dry season (analysed together). The significance tests of RDAs were done by analysis of variance (ANOVA). All analyses were in R environment (R Core Team 2017), with vegan (Oksanen et al. 2017), permute (Simpson 2016) and lattice (Deepayan 2008).

## Results

One hundred and thirty eight species were recorded in the plant community, 120 species in the native grassland (33 exclusive) and 103 species in the cultivated grassland dominated by *U. humidicola* (12 exclusive). Most species were in the Poaceae family (25 species), followed by Cyperaceae (16), Leguminosae (11), Asteraceae, Euphorbiaceae and Malvaceae (8 each), and Alismataceae (7). Together these represented over 50% of the species found in this community (Table S1). The pteridophytes *Isoetes pedersenii* and *Ophioglossum nudicaule* were counted in the seed bank.

Of the 120 species in the seed bank 50% were perennials and 50% annuals. Of the 110 species in the vegetation 40% were perennials and 60% were annuals. The richness of annual and perennial species in the seed bank between native and cultivated grasslands did not differ (Fig. 2a). The abundance of annual species in the seed bank was significantly higher (native: 888.09 seeds, cultivated: 812.41 seeds) compared to perennials (native: 414.87, cultivated: 436.42 seeds) ( $F=423.333$ ,  $p<0.001$ , Fig.2b), however, did not differ between native and

cultivated grasslands ( $F= 28.128$ ,  $p= 0.341$ , Fig. 2b). In the vegetation, annual species had higher richness in native grassland (51 spp.), differing in richness as for perennial species ( $F= 62.10$ ,  $p<0.001$ , Fig.2c) and grassland (Anova:  $F= 50.59$ ,  $p<0.001$ , Fig. 2c). For vegetation cover, we did not detect differences between annuals and perennials species ( $F= 6.135$ ,  $p= 0.0234$ , Fig. 2d) or grassland ( $F= 11.560$ ,  $p=0.351$ , Fig. 2d).

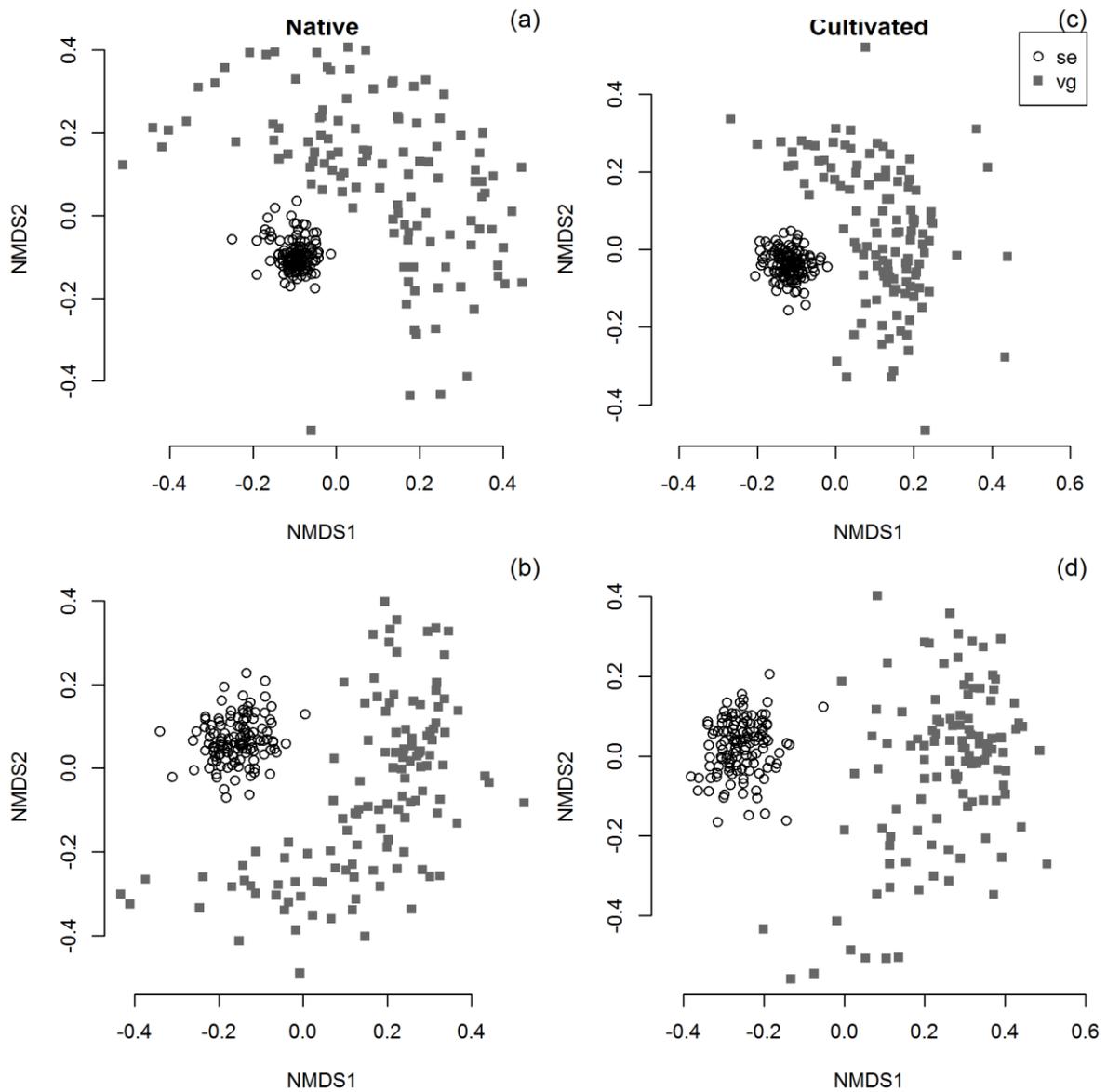


**Fig 2** Occurrence of annual and perennial species regarding richness (a) and mean abundance of seeds (b), species richness (c) and mean vegetation cover (d), between native and cultivated grassland in the seed bank and in the vegetation, in the Pantanal wetland (Central-West Brazil).

#### Similarity between seed bank and vegetation

The similarity between the abundance and relative percent cover of the seed bank and the vegetation in the native and cultivated grassland was under 50% when the seasonality was

analyzed separately. In flood and dry season it was 49% and 47% in native grassland and, 39% and 27% in cultivated grassland, respectively (Fig. 3).



**Fig 3** Ordination by non-metric multidimensional scaling (NMDS) of similarity de species (Bray-Curtis index), calculated from the abundance and relative cover of species no seed bank (se) and in the vegetation (vg), in native grassland em (a) flood and (b) dry season and grassland dominated by *U. humidicola* in (c) flood and (d) dry season, in the Pantanal wetland (Central-West Brazil).

In the flood season, seed bank composition differed from the vegetation in both the native ( $R^2=0.0866$ ,  $p<0.001$ , Fig. 3a) and cultivated grasslands ( $R^2= 0.1725$ ,  $p<0.001$ , Fig. 3b). In both grasslands there was higher species diversity in the seed bank than in the vegetation (Table 1). The individual contribution of each species in the similarity between the seed bank and the vegetation was below 2%. In the native grassland, *Bacopa myriophylloides* (1.54%), *Rotala ramosior* (1.32%), *Bacopa australis* (1.21%) and *Helanthis tenellum* (1.02%), were most similar species between vegetation and seed bank. And in cultivated, *R. ramosior* (1.92%), *Cyperus surinamensis* (1.33%), *Lipocarpa micrantha* (1.30%) and the exotic *U. humidicola* (1.25%).

**Table 1** Shannon diversity index between the seed bank and the vegetation (SE  $\pm$  mean), in native and cultivated grassland in the rainy and the dry season. The differences between the seed bank and the vegetation were obtained by ANOVA and Tukey test.

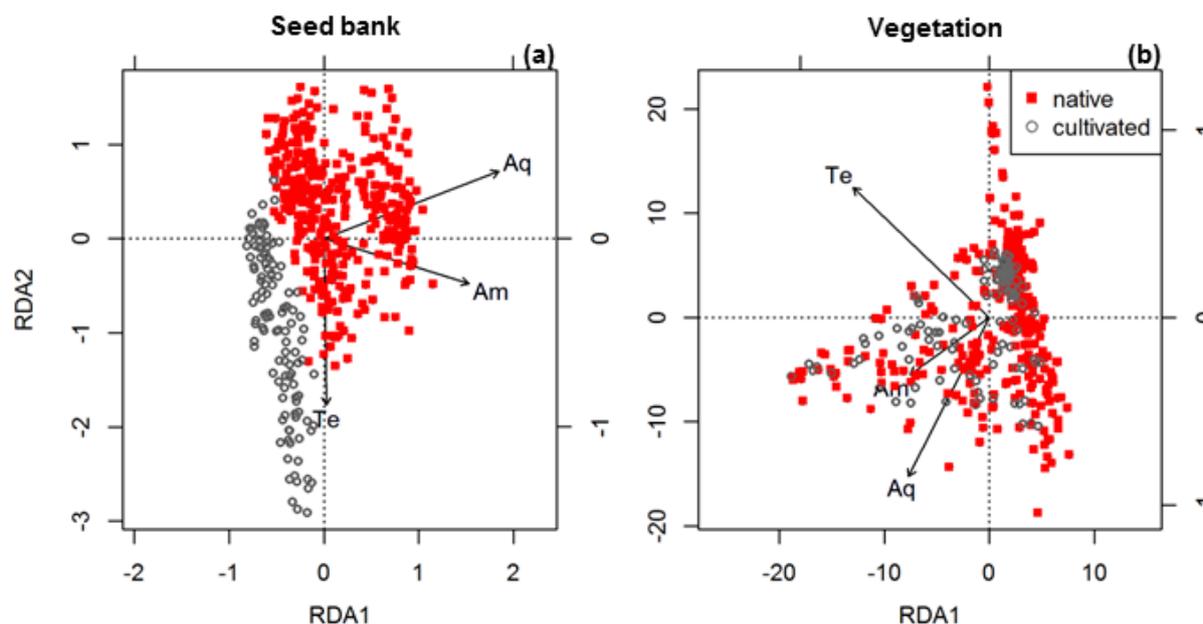
Grassland		Vegetation	Seed bank	F	P
Native	Season				
	flood	0.5060 $\pm$ 1.2559	0.4341 $\pm$ 2.7781	508	0.001
	dry	0.3896 $\pm$ 1.2721	0.4158 $\pm$ 2.4228	408	0.001
	flood+dry	0.7561 $\pm$ 1.8770	0.4514 $\pm$ 2.5773	876	0.001
Cultivated	flood	0.3420 $\pm$ 1.3826	0.4720 $\pm$ 2.6279	592	0.001
	dry	0.4089 $\pm$ 1.2411	0.3285 $\pm$ 2.3431	630	0.001
	flood+dry	0.7448 $\pm$ 1.9342	0.4225 $\pm$ 2.5605	962	0.001

In the dry season no similarity was detected between the seed bank and the vegetation in native ( $R^2= 0.08961$ ,  $p<0.001$ , Fig. 3c) and cultivated grassland ( $R^2= 0.09282$ ,  $p<0.001$ , Fig. 3d). The species which most similar the seed bank and the vegetation in native grassland were *Digitaria fuscescens* (7.1%), *Richardia grandiflora* (9.3%), *Axonopus purpusii* (9.8%) and *Borreria eryngioides* (10%), And in cultivated grassland, *Isoetes pedersennii* (5.2%), *U. humidicola* (5.5%), *Ludwigia octovalvis* (6.2%) and *Euploca filiformis* (7.1%).

To verify the absence of species that could be in the vegetation during the flood or dry season, a joint analysis of the seasons (dry + flood) was made, however, in spite of the seed bank and the vegetation having increased their similarity to 57% in native and 45% in cultivated grassland, they continued to differ significantly in both grasslands, native ( $R^2=0.45611$ ,  $p<0.001$ ) and cultivated ( $R^2=0.460$ ,  $p<0.001$ ). The index of species diversity increased in the seed bank and in the vegetation, nevertheless the diversity in the seed bank remained superior in both grasslands (Table 1), without effect of the seasonality in the above and bellowground differences.

#### Correlation of the growth forms with the seed bank and vegetation

Groups of plants were selected to explain how distinct growth forms (aquatic, amphibious and terrestrial) were related to species distribution between grasslands, in the seed bank and in the vegetation. The analyses revealed a clear split of native and cultivated grassland, in species composition of growth forms in the seed bank (Fig. 4a). According to redundancy analysis (RDA), there was a positive correlation in aquatic plants (41.3%) with distribution of species of native grassland (91.08 seeds), but negative with the cultivated grassland (52.96 seeds). The opposite occurred with terrestrial plants, positive for cultivated grassland (142.98 seeds) and negative for the native grassland (110.61 seeds). Aquatic and terrestrial plants were inversely correlated to both grasslands (Anova:  $F=17.883$ ,  $p<0.001$ , Fig. 4a). Nevertheless, in terms of diversity the seed bank was similar between the native and cultivated grassland (Table 1). The amphibious species were abundant in both grasslands, the species *Isoetes pedersenii* (13.1%), *Rotala ramosior* (12%), *E. minima* (10.4%), *Bacopa australis* and *Schoenoplectiella supina* (5% each), correlated positively (Fig. 4b).



**Fig 4** Redundance analysis (RDA), of the distribution of distinct growth forms in the (a) seed bank and (b) in the vegetation of the plots of native and cultivated grassland (Central-West Brazil). The ordination was based on data of abundance and relative cover of the species. Aq = aquatic; Am = amphibious and Te = terrestrial.

In RDA of the vegetation there was no separation of the grasslands, for the growth forms the occurrence of terrestrial plants (52.2%), was positively related to the axis RDA 2 including the higher composition of the plants present in cultivated grassland, differing from amphibious (28.7%) and aquatic plants (20.9%) (Anova:  $F=2.917$ ,  $p<0.001$ , Fig. 4c). The occurrence of the grasses *U. humidicola* (15.2%), *Axonopus purpusii* (9.3%), *Digitaria fuscescens* (7.4%), *Reimarochloa acuta* (6.6%), *Cynodom dactylon* (6.1%), *Paspalum alnum* (5.5%) and of the emergent *Richardia grandiflora* and *Diodia kuntzei* (5% each), *Hyptis brevipes*, *R. ramosior* and *Croton trinitatis* (4% each) were the most influent species in the distribution of the species enhancing the similarity between native and cultivated grassland by the group of terrestrial plants (Fig. 4d).

## Discussion

The characteristics of the vegetation and of the seed bank were studied in grasslands with native and exotic grasses. In these grasslands the predominance of annual species in the seed bank and in the vegetation indicates the high turnover that the seasonal environments promote in the vegetation, the cyclic effect can be the main source of similarity between the grasslands.

The higher diversity in the seed bank was also observed in other floodable grasslands, due to a large production of annual seeds tolerating the flood events (Brock et al. 2003, Ma et al. 2012), such tolerance of the seeds make this mechanism the main source of maintenance of the diversity of wetlands (Ge et al. 2013). In grasslands, the annual species by the fast colonization are the first to occupy the grassland at water reced (Bao et al. 2014), and next several native and exotic grasses to germinate (Bao et al. 2015). Yet in the rainy season, the aquatic plants which waiting for more moisture compose the vegetation (Agra and Ne'eman 2012). This dynamics of vegetations is only possible due to the persistent seed bank offering higher resilience to the environment (Brock 2011). Thereby, such areas under recurrent floods tend to present higher proportion of annual species with persistent seeds in the bank, and lower similarity with the established vegetation.

The lower richness in the vegetation can be due to the high mean plant cover of perennial grasses in native grassland (ca. 55%) and cultivated with dominance of *U.humidicola* (ca. 70%). In grasslands the dominance of a species leads to reduction in the germination of species with germinative characteristics below the dominant (Myers et al. 2005). This fact can explain the higher richness of annual plants in the vegetation, once such species are initial colonizers (Scott and Morgan 2012), present fast germination and reproduction (Dudney et al. 2016), independent on the stressing environmental conditions (McPeck and Peckarsky 1998). Yet the perennial species tend to have late germination and present low seed production (Gillespie and Volaire 2017), what caused low abundance of these species in the seed bank and in the vegetation.

## Similarity between the seed bank and the vegetation

Our results show that regardless of the dominance of *U.humidicola* in the cultivated grassland, the bank and the vegetation are not similar in both fields. The distinct communities of the standing vegetation and seed bank in the grasslands reflect the dispersal of seeds from nearby areas via hydrochory by secondary dispersal (Royo and Ristau 2013), the higher diversity of the seed bank in the rainy season assures the survival of several growth forms, once the seed bank can act as stabilizer in seasonal environments, assuring a vegetation in the flood and another in the drought (Combroux and Bornette 2004).

Seed banks is known to contain more species and greater diversity of species than established vegetation (Touzard et al. 2002, Ma et al. 2012, Yang and Li 2013), as they accumulate seeds for many years (Holzel and Otte 2004), with the longevity of grassland seeds of wetlands is attributed to the species-specific characteristics germination requirements (van Leeuwen et al. 2014, Baskin and Baskin 2014).

Yet aboveground vegetation is limited by the effect of grazing (not evaluated in this study) and by environmental filters, such as flooding (Myers and Harms 2009), that promotes loss of species in certain times of the year (Bao et al. 2015). Several terrestrial species without tolerance to submersion drop out the vegetation during the flood season (Vojtkó et al. 2017), e.g., *Eragrostis articulata*, *E. bahiensis*, *Panicum repens* and *Paspalum alium* (present in the seed bank in the flood season, Table S1). And the opposite occurs in the dry season, when the mortality of strictly aquatic plants is due to an intolerance to dessication (Blindow et al. 2016), e.g., *Cabomba furcata*, *Hetheranthera limosa* and *Limnocharis flava* (abundant in the seed bank in the dry season, Table S1). In this grassland, the trade-off in species between seasons only occurred in the vegetation, whereas the seed bank had a similar species composition in both seasons. The seed bank can remain with high richness and abundance for years, which can be a main source of regeneration in wetlands (Agra and Ne'eman 2012).

The differences between vegetation and seed bank are maintained by the higher diversity of the seed bank. The seed bank is an efficient regeneration mechanism rich in annual and perennial species that may not exhaust by germination, which is conferred to wet habitats by the species turnover from the dynamics of flood and drought (Capon et al. 2015). This way, the seed bank acts as a great receptor of species (in come of seeds) and the vegetation reflects a small part of the community hidden in the seed bank according to the environmental conditions, giving wet grasslands the necessary resilience to withstand the flood and the competition with exotic plants.

#### Correlation of the seed bank, seedlings and vegetation in relation to growth forms

The positive correlation of the seeds of aquatic plants in native grassland can indicate that evolutionarily these species are already adapted to the competition with the existing species in the seed bank. The similarities in requirements of resources for germination become favorable (McPeck and Peckarsky 1998). In addition to the presence of aquatic plants in the vegetation that increases the in come of seeds by dispersal and, consequently, increase the abundance of the seed bank of macrophytes (Metzner et al. 2017).

The lower abundance in the bank of aquatic plants in cultivated grassland, may indicate a low flow of these species due to failures in dispersal of nearby grasslands (Miao et al. 2016), or for low cover of these species in the vegetation, what reduces the income from seed rain (Auffret et al. 2017). The dominance of exotic grass in the vegetation can suppress growth of submerged macrophytes and of leaves of free floating, due to the reduction of light and nutrients, and can increase soil temperature (Gopal and Goel 1993, O'ahel'ová et al. 2011). Furthermore, the seeds and aerial parts of *U. humidicola* present allelopathic activity (Souza Filho et al., 2005), as well as observed in rice cultures, where the water extract of roots, stems leaves inhibits the germination of *Heteranthera limosa* (Ebana et al. 2001).

The lower abundance of aquatic plants in grassland is concerning, since they are economically utilized in the control of exotic plants for their capacity to tolerate either flooded or

dry soils, in the form of amphibious plants (Barrett et al. 1993), their distinct growth forms gives them high ability of colonization (Carrillo et al. 2017), yet, the role of the seed bank in the communities of aquatic plants still presents gaps in research, then although it has been demonstrated that most species are able to produce seeds, the vegetative propagation is its main mechanism of colonization (Grace 1993), this ability can lead many species to reduce their seed production over time (Charpentier 2001). However, despite lower abundance of aquatic plants in this grassland, this did not alter the diversity of the seed in the cultivated grassland which remained as diverse as the native.

The positive correlation of terrestrial plants in the vegetation and negative for aquatic plants as a whole, could be effect of sampling, once there was no stagnant water during a drought and little during samplings at flood, some submerged species did nor survive in the vegetation in the sampling period, increasing the negative correlation of aquatic plants in the aboveground vegetation, e.g., *Cabomba furcata*, *Hetheranthera limosa*, *Hydrocleys parviflora*, *Limnocharis flava*, *Nymphaea amazonum* and *N. gardneriana* were present in the seed bank and absent in the vegetation.

The homogeneous distribution in the aboveground vegetation between the native and cultivated grasslands indicate that the seed bank acts together with the dynamics of flood and drought, regulating the in come and exit de species. This composition and distribution vary according to space and time, and patterns of flood and drought (Brock et al. 2003). The form how the dynamics of flood and drought acts on the distribution of the speciess of the seed bank and of the vegetation can provide information that allow the prediction of the consequences of the introduction of exotic grasses in this grassland, the change in water regime, with drier years can imply in increase of dominance of *U. humidicola* and loss of the aquatic community.

## Conclusion

To identify the strategies de adaptation of plants and their responses to the environmental pressures (flood and drought) and human activity (introduction of exotic species), render these environment highly selective, to understand the distribution and the ecological needs for survival and regeneration of these communities are fundamental for formulation of sustentainable use of the environment and conservation of the wetlands. Our results indicate that the presence of *U. humidicola* has to be treated with care, such exotic grasses tend to suppress other herbs and over the years can lead to loss of diversity in the bank and consequently in the vegetation.

The seed bank demonstrated potential to predict the species richness and abundance of the grassland. With the study of the distribution of growth forms we can observe where does occur reduction in the abundance of seeds of some species, which possibly are not strong competitors in presence of the dominant species. The flood period acts in maintenance of the diversity of this community, where most species are adapted to conditions of flood and drought, as observed, there is higher richness and abundance of amphibious plants in the seed bank and in the vegetation, in both grasslands (Table S1), what approximates the diversity of native and cultivated grassland. This can become particularly important in the formulation of future plans of conservation and preservation of the wetland.

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## **Supplementary Material**

**Table S1** List of species sampled in the soil seed bank (values of total abundance per sample) and in the vegetation (% mean cover per sample) in native (N) and cultivated grassland (C) during the wet and dry season, classified regarding life cycle (annual - A and perennial plants - P) and growth forms (Aquatic - Aq, amphibious - Am and terrestrial - T), in the sub-region Abobral in the Pantanal wetland, MS, Brazil.

	Life cycle	Growth forms	Seed bank (n°)				Vegetation cover (%)			
			Field	Dry	Field	Flood	Field	Dry	Field	Flood
<b>ACANTHACEAE</b>										
<i>Justicia laevilinguis</i> (Nees) Lindau	P	T	-	-	-	-	N	2.1	N	2.4
<b>ALISMATACEAE</b>										
<i>Echinodorus glaucus</i> Rataj	P	Aq	-	-	-	-			N	2.8
<i>E. grandiflorus</i> (Cham. & Schltld.) Micheli	P	Aq	N/C	171	N/C	274	N	2	N/C	15.9
<i>E. longiscapus</i> Arechav.	P	Aq			N	7	N/C	2	N/C	8.9
<i>Helanthium tenellum</i> (Martius) Britton	P	Aq	N/C	2521	N/C	3245	-	-	N/C	24.1
<i>Hydrocleys parviflora</i> Seub.	P	Aq	N/C	1012	N/C	1144	-	-	N	13.2
<i>Limnocharis flava</i> (L.) Buchenau	P	Aq	N/C	2627	N/C	1053	-	-	-	-
<i>Sagittaria guayanensis</i> Kunth	P	Aq	N/C	868	N/C	1471		-	N/C	16.1
<b>AMARANTHACEAE</b>										
<i>Pfaffia glomerata</i> (Spreng.) Pedersen	P	T	-	-	-	-	N/C	3	-	-
<b>APIACEAE</b>										
<i>Eryngium ciliatum</i> Cham. & Schltld.	A	T	N/C	6	N/C	171	-	-	-	-
<b>APOCYNACEAE</b>										
<i>Metastelma berterianum</i> (Spreng.) Decne.	P	T	-	-	-	-	N	9.2	-	-
<b>ASTERACEAE</b>										
<i>Baccharis glandulosa</i> Greenm.	P	Am	N	8	-	-	-	-	-	-
<i>Conyza bonariensis</i> (L.) Cronquist	A	T	-	-	N/C	111	N	6.2		
<i>Erechtites hieraciifolius</i> (L.) Raf. ex DC.	A	T	N/C	13	-	-	-	-	-	-
<i>Gamochoeta purpurea</i> (L.) Cabrera	A	T	N/C	16	-	-	-	-	-	-
<i>Praxelis clematidea</i> R.M. King & H. Rob.	A	T	N/C	40	N/C	444	N/C	15.7	C	11.2
<i>Sphagneticola brachycarpa</i> (Baker) Pruski	P	Am	N/C	55	N/C	5425	N/C	11.8	N	4.9
<i>Stilpnopappus pantanalensis</i> H. Rob.	A	T	N/C	414	N/C	2274	N/C	13.2	N/C	10.6
<i>Vernonanthura brasiliiana</i> (L.) H. Rob.	P	T	N/C	31	C	3	-	-	-	-
<b>CABOMBACACEAE</b>										

<i>Cabomba furcata</i> Schult. & Schult. f.	A	A	N/C	32	N/C	26	-	-	N	9.1
<b>COMMELINACEAE</b>										
<i>Commelina erecta</i> L.	P	T	N	2	N/C	74	-	-	-	-
<b>CONVOLVULACEAE</b>										
<i>Aniseia martinicensis</i> (Jacq.) Choisy	P	T	-	-	-	-	N/C	17.2	-	-
<i>Ipomoea subrevoluta</i> Choisy	P	Am	-	-	-	-	N/C	15.3	-	-
<b>CYPERACEAE</b>										
<i>Cyperus compressus</i> L.	P	T	N/C	800	N/C	239	-	-	-	-
<i>C. cornelii-ostenii</i> Kük.	P	T	N/C	174	N/C	285	N/C	7.9	N	6.2
<i>C. haspan</i> L.	P	Am	N/C	2059	N/C	2250	N/C	15.2	N/C	17.5
<i>C. lanceolatus</i> Poir.	P	T	N/C	82	N/C	41	-	-	-	-
<i>C. surinamensi</i> Rottb.	A	Am	N/C	1816	N/C	2571	N/C	21.8	N/C	11.9
<i>Eleocharis acutangula</i> (Roxb.) Schult.	P	Aq	N/C	3295	N/C	1569	-	-	N/C	18.7
<i>E. filiculmis</i> Kunth	P	Aq	N/C	248	-	-	-	-	-	-
<i>E. geniculata</i> (L.) Roem. & Schult.	P	Aq	N/C	12	N/C	10	-	-	-	-
<i>E. minima</i> Kunth	A	A	N/C	2260	N/C	3059	N/C	10.6	N/C	14.7
<i>Fimbristylis</i> sp.	A	T	N/C	52	N/C	350	-	-	-	-
<i>Fimbristylis dichotoma</i> (L.) Vahl	A	T	N/C	1949	N/C	1414	N/C	15.9	-	-
<i>Killinga brevifolia</i> Rottb.	P	Am	NC	144	N/C	1501	N/C	8.1	N/C	7.9
<i>Lipocarpa micrantha</i> (Vahl) G.C. Tucker	A	T	N/C	587	N/C	540	-	-	-	-
<i>L. humboldtiana</i> Nees	P	Am	N/C	1681	N/C	452	-	-	-	-
<i>Schoenoplectiella supina</i> (L.) Lye	P	Aq	N/C	1991	N/C	3443	-	-	N	8.5
<b>EUPHORBIACEAE</b>										
<i>Caperonia castaneifolia</i> (L.) A. St.-Hil.	P	Am	N/C	80	N/C	38	N/C	16.2	N	15.1
<i>C. palustris</i> (L.) A. St.-Hil.	P	T	-	-	-	-	-	-	N	5.2
<i>Croton glandulosus</i> L.	A	T	-	-	-	-	N/C	16		
<i>C. trinitatis</i> Millsp.	A	T	N/C	862	N/C	1667	N/C	25.4	N/C	15.2
<i>Euphorbia hirta</i> L.	A	T	N/C	9	N/C	1141	N/C	18	-	-
<i>E. hyssopifolia</i> L.	A	T	N/C	16	N/C	11	N	7	-	-
<i>Euphorbia thymifolia</i> L.	A	T	N/C	1876	N/C	1126	N/C	5	-	-

<i>Microstachys hispida</i> (Mart.) Govaerts	P	T	N/C	243	N/C	480	N/C	31.9	N/C	20.1
<b>GENTIANACEAE</b>										
<i>Schultesia guianensis</i> (Aubl.) Malme	A	T	N	5	N	4	-	-	-	-
<b>HELIOTROPIACEAE</b>										
<i>Euploca filiformis</i> (Lehm.) J.I.M.Melo & Semir	A	T	N/C	1671	N/C	2186	N/C	12	-	-
<i>E. procumbens</i> (Mill.) Diane & Hilger	A	T	N/C	123	N/C	52	-	-	-	-
<i>Heliotropium indicum</i> L.	A	T	C	6	C	2	-	-	-	-
<b>HYDROCHARITACEAE</b>										
<i>Najas</i> L.	A	Aq	N	8	-	-	-	-	-	-
<b>HYDROLEACEAE</b>										
<i>Hydrolea spinosa</i> L.	P	T	-	-	-	-	N	5.8	-	-
<b>ISOETACEAE</b>										
<i>Isoetes pedersenii</i> H.P. Fuchs ex Hickey	P	Am	N/C	20152	N/C	15761	N/C	15.1	C	16.2
<b>LAMIACEAE</b>										
<i>Hyptis brevipes</i> Poit.	A	T	N/C	6072	N/C	4395	N/C	16.7	N/C	15.4
<i>H. lorentziana</i> O. Hoffm.	A	T	N/C	288	N/C	352	N/C	8.2	N/C	11.1
<b>LEGUMINOSAE</b>										
<i>Aeschynomene fluminensis</i> Vell.	P	Am	-	-	-	-	C	4.2	-	-
<i>A. histrix</i> Poir	P	Am	N/C	12	-	-	-	-	-	-
<i>Chamaecrista serpens</i> (L.) Greene	P	T	-	-	-	-	N	13.8	-	-
<i>Crotalaria micans</i> Link	P	T	N/C	123	N/C	187	-	-	-	-
<i>Discolobium pulchellum</i> Benth.	P	T	-	-	-	-	-	-	N	4.4
<i>Indigofera lespedezioides</i> Kunth	P	T	-	-	-	-	N	3.2	-	-
<i>Mimosa pigra</i> L.	P	T	-	-	-	-	N/C	11.4	N	10.5
<i>M. weddeliana</i>	P	T	N/C	7	C	2	N/C	15.3	-	-
<i>Senna aculeata</i> (Pohl ex Benth.) H.S. Irwin & Barneby	P	T	C	8	-	-	N	10.4	-	-
<i>S. obtusifolia</i> (L.)H.S.Irwing& Barneby	P	T	N	7	-	-	-	-	-	-
<i>S. occidentalis</i> (L.)Link	P	T	N	2	N/C	11	-	-	C	5.3
<b>LYTHRACEAE</b>										
<i>Cuphea retrorsicapilla</i> Koehne	A	T	-	-	-	-	C	5.4	N/C	3.3

<i>Rotala ramosior</i> (L.) Koehne	A	Am	N/C	2863	N/C	5435	N/C	7.5	N/C	18.1
<b>MALPIGHIACEAE</b>										
<i>Byrsonima cydoniifolia</i> A. Juss.	P	T	-	-	-	-	N/C	15.7	N	10.2
<b>MALVACEAE</b>										
<i>Melochia arenosa</i> Benth.	P	T	-	-	-	-	N	10.1	N/C	6.9
<i>M. parviflora</i> Loudon	A	T	-	-	-	-	N	2.6	-	-
<i>M. simplex</i> A. St.-Hil.	P	T	N/C	91	N/C	410	N/C	18.3	N/C	12.6
<i>M. villosa</i> (Mill.) Fawc. & Rendle	P	T	-	-	-	-	N/C	4.8	-	-
<i>Sida cerradoensis</i> Krap.	A	T	N/C	662	N/C	618	N/C	5.1	C	4.2
<i>S. rhombifolia</i> L.	P	T	N/C	162	N/C	18	N/C	7.4	N	5.3
<i>S. viarum</i> A. St.-Hil.	P	T	N/C	16	N/C	104	-	-	-	-
<b>NYMPHAEACEAE</b>										
<i>Nymphaea amazonum</i> Mart. & Zucc.	P	Aq	N/C	117	N/C	201	-	-	-	-
<i>N. gardneriana</i> Planch.	P	Aq	N/C	274	N/C	418	-	-	-	-
<b>OCHNACEAE</b>										
<i>Sauvagesia erecta</i> L.	A	T	N	22	N	7	-	-	-	-
<b>ONAGRACEAE</b>										
<i>Ludwigia grandiflora</i> (Michx.) Greuter & Burdet	P	Am	N/C	67	N/C	94	N/C	3.1	N	3.8
<i>L. inclinata</i> (L. f.) M. Gómez	A	Am	N/C	49	N/C	73	N	2.7	N/C	3.6
<i>L. octovalvis</i> (Jacq.) P.H. Raven	A	Am	N/C	3295	N/C	4810	N/C	11.9	N/C	16.2
<b>OPHIOGLOSSACEAE</b>										
<i>Ophioglossum nudicaule</i> L. f.	A	Am	N/C	26	N/C	229	C	5.6	-	-
<b>OROBANCHACEAE</b>										
<i>Agalinis glandulosa</i> (G.M.Barroso) V.C. Souza	A	T	N/C	34	N/C	82	N/C	4.2	-	-
<i>Buchnera longifolia</i> Kunth	P	T	-	-	-	-	N	2.4	-	-
<b>PHYLLANTHACEAE</b>										
<i>Phyllanthus stipulatus</i> (Raf.) G.L. Webster	P	T	N/C	29	N/C	60	N	6.7	N	5.5
<i>P. orbiculatus</i> Rich.	P	T	N/C	12	N/C	14	-	-	-	-
<b>PLANTAGINACEAE</b>										
<i>Angelonia salicariifolia</i> Bonpl.	P	Am	N/C	328	N/C	589	N/C	7.5	N/C	8.6

<i>Bacopa australis</i> V.C. Souza	P	A	N/C	1761	N/C	2728	N/C	2.8	N	15.1
<i>B. myriophylloides</i> Wettst.	P	A	N	9	N/C	115	C	5.3	N/C	4.9
<i>B. salzmännii</i> (Benth.) Wettst. ex Edwall	P	A	N/C	413	N/C	348	-	-	N	8.1
<i>B. stricta</i> (Schrad.) Edwall	A	Am	N/C	53	N/C	64	-	-	N	6.2
<i>Scoparia dulcis</i> L.	A	T	N/C	25	N/C	255	-	-	-	-
<i>S. montevidensis</i> (Spreng.) R.E. Fr.	A	T	N/C	824	N/C	1614	N/C	10.8	N/C	7.2
<b>POACEAE</b>										
<i>Andropogon hypogynus</i> Hack.	P	T	-	-	-	-	C	10.2	C	7.3
<i>Axonopus leptostachyus</i> (Flüggé) Hitchc.	P	T	-	-	-	-	N/C	28.9	N/C	14.2
<i>A. purpusii</i> (Mez) Chase	P	T	N/C	834	N/C	832	N/C	44.3	N/C	40.3
<i>Cynodon dactylon</i> (L.) Pers.	P	T	N/C	9	N/C	735	N/C	26.2	N/C	17.2
<i>Digitaria bicornis</i> (Lam.) Roem. & Schult.	P	T	N/C	254	N/C	557	C	15.3	C	10.2
<i>D. ciliaris</i> (Retz.) Koeler	P	T	N/C	232	N/C	99	N/C	22.1	-	-
<i>D. fuscescens</i> (J. Presl) Henrard	P	T	-	-	N/C	992	N/C	52.1	N/C	26.4
<i>D. horizontalis</i> Willd.	P	T	-	-	-	-	N/C	10.6	-	-
<i>Eragrostis articulata</i> (Schrank) Nees	A	T	N/C	14	N/C	37	-	-	-	-
<i>E. bahiensis</i> Schrad. ex Schult.	A	T	N	6	N	9	N/C	14.2	-	-
<i>Echinochloa polystachya</i> (Kunth) Hitchc	P	T	-	-	-	-	N/C	3.5	-	-
<i>Hemarthria altissima</i> (Poir.) Stapf & C.E. Hubb.	P	T	N	5	N	6	N/C	20.1	-	-
<i>Leersia hexandra</i> G.C. Tucker	P	T	N/C	10	N/C	12	N/C	25.3	C	12.3
<i>Panicum dichotomiflorum</i> Michx.	P	T	N/C	222	N/C	114	-	-	-	-
<i>P. repens</i> L.	P	T	-	-	-	-	N	10.1	-	-
<i>Paspalum alnum</i> Chase	P	T	N/C	42	N/C	447	N/C	31.2	-	-
<i>P. notatum</i> Alain ex Flüggé	P	T	-	-	-	-	N	5.9	-	-
<i>P. oteroi</i> Swallen	P	T	N/C	434	-	-	N	10.8	-	-
<i>P. plicatulum</i> Michx.	P	T	-	-	N/C	192	N	4.8	-	-
<i>Pennisetum glaucum</i> (L.) R. Br.	P	T	-	-	C	81	C	11.9	C	6.4
<i>Reimarochloa acuta</i> (Flüggé) Hitchc.	P	T	N/C	36	N/C	43	N/C	41.2	N/C	32.1
<i>Setaria parviflora</i> (Poir.) Kerguélen	A	T	N/C	1462	N/C	1453	N/C	18.3	N/C	14.2
<i>Steinchisma laxum</i> (Sw.) Zuloag	A	T	N/C	330	N/C	295	-	12.4	N/C	10.3

<i>Urochloa humidicola</i> (Rendle) Morrone & Zuloaga	P	T	N/C	505	N/C	341	C	59.4	C	25.6
<b>POLYGALACEAE</b>										
<i>Polygala timoutoides</i> Chodat	A	T	N	12	-	-	-	-	-	-
<i>P. leptocaulis</i> Torr. & A. Gray	A	Am	N/C	87	N/C	29	C	11		
<i>P. molluginifolia</i> A. St.-Hil. & Moq.	A	Am	N/C	55	N/C	180	N/C	15.4	C	12.5
<b>POLYGOLACEAE</b>										
<i>Polygonum acuminatum</i> Kunth	P	Am	N/G	15	-	-	-	-	-	-
<i>P. punctatum</i> Elliott	P	Am	N/C	35	-	-	N	8	-	-
<b>PONTEDERIACEAE</b>										
<i>Heteranthera limosa</i> (Sw.) Willd.	P	Aq	N/C	63	N/C	230	-	-	N	12.1
<i>Pontederia parviflora</i> Alexander	P	Aq	N/C	45	N/C	60	-	-	N	6.4
<i>P. subovata</i> (Seub.) Lowden	P	Aq	N/C	285	N/C	623	-	-	N/C	12.3
<b>PORTULACACEAE</b>										
<i>Portulaca fluvialis</i> D. Legrand	P	T	N/C	54	N/C	157	-	-	-	-
<i>P. pilosa</i> L.	A	T	N/C	770	N/C	723	N	6.6	-	-
<b>RUBIACEAE</b>										
<i>Borreria eryngioides</i> Cham. & Schltldl.	A	Am	N/C	120	N/C	292	N	7.2	-	-
<i>Diodia kuntzei</i> K. Schum.	P	T	N/C	958	N/C	1662	N/C	37.1	N/C	11.2
<i>Richardia grandiflora</i> (Cham. & Schltldl.) Steud.	A	T	N/C	2446	N/C	6516	N/C	31.9	N/C	19.3
<i>Spermacoce glabra</i> Michx.	P	T	N/C	18	N/C	13	N	5.6	-	-
<i>Staelia thymoides</i> Cham. & Schltldl.	A	Am	N/C	40	N/C	20	N/C	7.1	N/C	12.4
<b>TURNERACEAE</b>										
<i>Turnera melochioides</i> Cambess.	A	T	N/C	67	N/C	385	N/C	6.2	-	-
<b>VERBENACEAE</b>										
<i>Lippia alba</i> (Mill.) N.E. Br. ex Britton & P. Wilson	P	T	C	4	C	3	N	8.6	-	-

## Considerações finais

Os resultados aqui levantados reforçam a importância da utilização do estudo do banco de sementes como principal fonte indicadora das espécies potenciais de regeneração e manutenção da vegetação em comunidades de áreas úmidas. Os campos de pastagem são objeto de estudo pela recorrente utilização na atividade agropecuária, o que leva a introdução de espécies exóticas em todo o mundo, no Pantanal a introdução de *Urochloa humidicola* é uma ameaça pela alta tolerância à inundação e rápida propagação, o que pode levar a perda de diversidade. Os eventos de inundação podem atuar de maneira negativa transportando estas sementes para diversas áreas vizinhas, como também atuar de forma positiva, aumentando a abundância e riqueza de espécies nativas, selecionando espécies adaptadas à inundação, que possam competir com a espécie exótica.

O objetivo principal desta tese foi avaliar as estratégias de colonização da *U. humidicola* e, como as espécies nativas atuam no controle de invasão, tendo a favor os eventos de inundação. Desta forma, no primeiro capítulo, abordamos a vegetação adulta em campo, as diferenças observadas na vegetação acima do solo entre as pastagens nativas e cultivadas foram indícios da dominância de *U. humidicola* na vegetação no período de seca, contudo, o aumento da similaridade entre as pastagens no período de pós-inundação revelou uma comunidade nativa adaptada aos eventos sazonais e com alto potencial competidor. Essas plantas nativas regeneram-se rapidamente, principalmente, devido à riqueza e abundância local de espécies emergentes e gramíneas nativas. Contudo, a diminuição da cobertura aérea de *U. humidicola* após a inundação e a recolonização no período de seca, nos levou ao segundo capítulo.

Neste segundo capítulo, tivemos em vista a questão orientadora de como estes campos de pastagem do Pantanal persistem durante anos de introdução de espécies exóticas em locais onde as condições de inundação podem favorecer a dispersão de sementes de espécies exóticas. Através de um estudo experimental apoiamos nossas pesquisas de campo, onde avaliamos se a mortalidade de *U. humidicola* durante a inundação estava associada a presença das espécies

nativas (macrófitas aquáticas) e, avaliamos a persistência do banco de sementes da *U. humidicola* e suas estratégias germinativas. Nossos resultados revelaram que a população adulta de *U. humidicola* pode ser controlada pela ocorrência de macrófitas aquáticas, que durante as inundações formam extensos tapetes que diminuem a incidência de luz e levam a perda de cobertura aérea de *Urochloa*, contudo salientamos a importância de estudos que visem o mecanismo de rebrota que deve ser controlado, assim como, o banco de sementes, que em nosso estudo mostrou alto potencial de germinação e colonização.

Desta forma, no terceiro capítulo, avaliamos o potencial do banco como fonte indicadora das espécies nativas que se estabelecem durante e após o evento extremo sazonal, a rotatividade de espécies que ocorre dentro de um curto espaço de tempo favorece as espécies nativas. O ambiente quando inundado apresenta um rico banco de sementes de espécies aquáticas e anfíbias e, na pós-inundação, ocorre uma explosão de anfíbias anuais e terrestres, no decorrer do tempo muitas gramíneas dão início a germinação. Essa resposta diferencial entre as espécies em função do ambiente ajuda a manter a estrutura e riqueza de espécies na comunidade. Também destacamos a importância de que as amostragens em pastagens inundáveis sejam realizadas em diferentes estações e a avaliação do banco de sementes em pelo menos dois métodos: simulando inundação e seca, para uma melhor avaliação da comunidade.

O processo de regeneração em campo foi avaliado no quarto capítulo, onde estudamos a distribuição do banco de plântulas sob influência dos detritos orgânicos que são formados devido ao aumento de macrófitas aquáticas durante a inundação e, que morrem no rebaixamento das águas junto às espécies terrestres que não toleram o alagamento, estes detritos cobrem todo campo de pastagem em diferentes concentrações de acordo com a topografia. Nossos resultados revelaram que a distribuição de plântulas varia de acordo com as características germinativas de cada espécie em função da topografia e quantidade de detritos, os resultados apresentados indicaram que a inundação reflete no potencial de regeneração e é uma importante força seletiva que impulsiona as estratégias individuais das espécies para colonização bem sucedida.

E por fim, no último capítulo correlacionamos o banco de sementes e a vegetação para identificar as estratégias de adaptação das plantas e suas respostas às pressões ambientais (inundação e seca) e a introdução de espécies exóticas. O banco de sementes apresentou potencial para manter a diversidade de espécies nativas nesta comunidade, uma vez que o ciclo de inundações e secas promoveu a entrada de espécies distintas no banco, a menor riqueza na vegetação reflete a dependência das características germinativas de cada espécie em função da sazonalidade. Nossos resultados indicam que a presença de *U. humidicola* deve ser tratada com cuidado devido à redução do banco de macrófitas nas pastagens cultivadas, uma vez que essas espécies podem atuar no controle de plantas exóticas. O período de inundação atua na manutenção da diversidade desta comunidade, onde a maioria das espécies é adaptada às condições de inundação e seca, como observado, a maior riqueza e abundância de plantas anfíbias no banco e na vegetação, em ambas as pastagens, mantém próxima a diversidade de pastagens nativas e cultivadas. Isso pode se tornar particularmente importante na formulação de futuros planos de conservação e preservação da zona úmida.