Universidade de Brasília
Instituto de Ciências Biológicas
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Combination of techniques to control invasive grasses and restore Brazilian savannah - steps to achieve large-scale restoration

Augusto Cesar Silva Coelho

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Combination of techniques to control invasive grasses and restore Brazilian savannah - steps to achieve large-scale restoration

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Resumo

A presença de espécies exóticas invasoras é um dos principais obstáculos para a restauração ecológica. Este obstáculo parece ser ainda mais indiscutível em ecossistemas abertos, como campos e savanas, dada a dificuldade de controle de gramíneas exóticas invasoras (GEI). O controle efetivo de GEI é fundamental para que o Brasil cumpra as metas de restauração em larga escala estabelecidas em acordos internacionais. Neste estudo, foram testadas técnicas de controle mecânico aliadas ao controle químico de GEI, associando fogo, gradagens de solo e semeadura direta de espécies nativas para contribuir com a restauração de uma área antes dominada por *Urochloa decumbens* (numa área de solo bem drenado) e outra área dominada por *U. humidicula* (em uma área sazonalmente inundada). As técnicas de controle aplicadas foram eficazes para reduzir a cobertura de GEI. A semeadura direta foi especialmente eficaz na área bem drenada, aumentando a cobertura de espécies nativas. No entanto, essas intervenções também levaram a um aumento na cobertura de espécies ruderais espontâneas que podem direcionar a comunidade para diferentes trajetórias sucessionais. A sucessão da comunidade de plantas deve ser avaliada em longo prazo para uma melhor compreensão dos resultados dessas intervenções. Nós mostramos evidências iniciais de que o manejo integrado, com fogo, controle mecânico e químico, é um caminho promissor para alcançar a restauração em larga escala no Cerrado.

Palavras-chave: controle químico, manejo, ecossistema aberto, invasão de plantas, *Urochloa decumbens*, *Urochloa humidicula*.

Abstract

The presence of invasive non-native species is one of the main obstacles to ecological restoration. This obstacle seems to be even more indisputable in open ecosystems, such as grasslands and savannas, given the difficulty in controlling invasive grass species (IGS). The effective control of IGS is key for Brazil to fulfil large-scale restoration goals established in international agreements. In this study, we tested mechanical control techniques combined with chemical control of IGS in association with fire, soil harrowing, and direct seeding of native species to contribute to the restoration of an area previously dominated by *Urochloa decumbens* (well-drained area) and another area dominated by *U. humidicula* (waterlogged area). The control techniques applied were effective to reduce IGS cover. The reintroduction of native species by direct seeding was especially effective to increase the cover of native speciesin the well-drained area. However, these interventions also led to an increase in the cover of spontaneous ruderal species that may direct the community to different trajectories. Succession of the plant community must be assessed in the long term to better understand the results of these interventions. We showed initial evidence that integrated management, with fire, mechanical and chemical control of IGS, is a promising way forward to achievelarge-scale restoration in Brazilian savannah.

Keywords: chemical control, management, open ecosystem, plant invasion, *Urochloa decumbens, Urochloa humidicula*

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Introdução Geral

A transposição de barreiras geográficas e ecológicas possibilita que espécies introduzidas se tornem invasoras, sendo consideradas um fator de degradação de relevância global (D'Antonio & Vitousek, 1992; D'Antonio & Meyerson, 2002; Simberloff et al. 2013; Veldman et al., 2015; Da Silveira et al., 2018). Gramíneas exóticas com potencial invasor (GEI) estão distribuídas pelo globo, gerando impactos principalmente nos trópicos (Flórido, 2015; Weidlich et al. 2020). Em muitos casos, áreas dominadas por GEI não têm capacidade de retornar ao seu estado original mesmo após o abandono, requerendo restauração ativa com intervenções que acelerem o processo de restauração da área (Holl & Aide, 2011; Link et al. 2017; Barbosa et al. 2018; Cava et al. 2018). Para isso, o controle dessas GEI e a reintrodução de espécies nativas estão entre as práticas mais importantes para viabilizar a restauração em larga escala (D'Antonio & Meyerson, 2002; Zenni & Ziller, 2011; Thomas et al. 2018; Buisson et al. 2019; Schmidt et al. 2019).

Tem-se testado diferentes métodos de controle de GEI (Cava et al. 2016; Da Silveira et al. 2018; Buisson et al. 2019). O plantio de árvores nativas em alta densidade tem a capacidade de controlar GEI pelo sombreamento do solo (Cabin et al. 2002; Rodrigues et al. 2009; Flórido, 2015). Diferentemente do que é feito para a restauração de ambientes de dossel fechado, como florestas, o plantio de alta densidade de árvores não é recomendado em ambientes abertos, já que descaracterizaria a vegetação original e poderia eliminar grupos funcionais característicos da área, como gramíneas nativas (Parr et al. 2014; Veldman et al. 2015; Schmidt et al. 2019). Assim, o controle de GEI torna-se um desafio para a restauração de ambientes abertos (Buisson et al. 2019; Coutinho et al. 2019; Sampaio et al. 2019).

O uso do fogo (controle físico) diminui a biomassa das GEI e reduz a quantidade de sementes recém produzidas, diminuindo a dispersão e presença de propágulos na área, mas não é eficaz para o controle das GEI mais difundidas em pastagens (Martins et al. 2011; Sampaio et al. 2019), visto que , a maior parte das gramíneas evoluiu com e é adaptada ao fogo. Muitas vezes o benefício entre fogo e GEI é mútuo, com as GEI inflamáveis alterando o regime de fogo e as queimadas intensificadas degradando o ambiente, tornam-o mais suscetível a invasões biológicas (D'Antonio & Vitousek, 1992; Pivello et al. 1999; Lehmann et al. 2011; Buisson et al. 2019). Assim, o fogo não é, isoladamente, efetivo no controle de GEI (Martins et al. 2011; Bonello & Judd, 2020). Gradagens do solo podem eliminar GEI estabelecidas na área e expor as sementes presentes no banco de sementes, favorecendo sua germinação e posterior morte pelo subsequente revolvimento do solo (Andrade, 2019;

Sampaio et al. 2019). Porém, experimentos indicam que GEI são capazes de recolonizar áreas em processo de restauração mesmo quando nos primeiros anos há um aparente sucesso no controle (Pellizzaro et al. 2017; Andrade, 2019; Coutinho et al. 2019; Sampaio et al. 2019; Liaffa, 2020).

A aspersão de herbicidas é utilizada em rotações de plantios para integração lavoura-pecuária, onde a eliminação da cultura de gramínea introduzida para pastejo pode ser feita com uso do controle químico (Vilela et al. 2011). A aspersão também é utilizada para restauração de áreas de dossel aberto em regiões temperadas, controlando exóticas e beneficiando, indiretamente, a comunidade de plantas nativas (Bakker et al. 2003; Link et al. 2017; Bonello & Judd, 2020). Para o controle de GEI em áreas abertas na savana neotropical (Cerrado), bons resultados foram observados em projetos com manejo integrado, unindo o fogo e/ou gradagem do solo ao controle químico (Flórido, 2015; Andrade, 2019). O controle químico de plantas exóticas invasoras com uso de herbicidas proporciona a eliminação da barreira imposta por estas às espécies nativas, facilitando o processo de regeneração da área com custos financeiros mais baixos (Dechoum & Ziller, 2013; Flórido, 2015; Fragoso et al. 2017; Thomas et al. 2018). Experimentos com aplicação de herbicidas no manejo integrado em áreas abertas no Cerrado têm atestado a redução da cobertura de GEI, chegando a 72% de redução ao fim de uma estação chuvosa (Andrade, 2019; Santos, 2020). Mesmo com tais resultados, ainda há grande resistência ao uso de herbicidas em Unidades de Conservação de Proteção Integral (UCPI) no Brasil, não existindo até então nenhum estudo com manejo integrado com controle físico/mecânico/químico de GEI em UCPI.

Mesmo com um bom controle de GEI, a limitação de sementes nativas e a grande abundância de sementes exóticas podem dificultar a regeneração natural, sendo importante a reintrodução de espécies nativas (D’Antonio & Meyerson, 2002; Endress et al. 2012). Neste sentido, para a restauração de áreas campestres e savânicas, a semeadura direta é uma técnica capaz de reintroduzir simultaneamente plantas de diferentes formas de vida, permitindo que estas se estabeleçam no local (Andrade et al. 2015; Pellizzaro et al. 2017; Da Silveira et al. 2018; Buisson et al. 2019; Sampaio et al. 2019; Schmidt et al. 2019).

Em projetos de restauração, a semeadura direta de espécies nativas tem apresentado um melhor custo-benefício para introdução de espécies lenhosas em savanas do que o plantio de mudas (Raupp et al. 2020). Experimentos com semeadura de espécies do estrato rasteiro da vegetação podem apresentar altas taxas de estabelecimento e sobrevivência, contribuindo para o controle de espécies exóticas em regiões temperadas (Bakker et al. 2003; Endress et al. 2012). Além disto, a produção de mudas de gramíneas e herbáceas não é comum, e a densidade e quantidade requerida para restauração do estrato graminoso tende a ser logística e economicamente proibitiva (Schmidt et al. 2019; Raupp et al. 2020). Assim, a semeadura direta é a técnica com maior capacidade de suprir a demanda de restauração do estrato rasteiro, sendo recomendada para o Cerrado (Sampaio et al., 2015; Pellizzaro et al. 2017; Andrade, 2019; Coutinho et al. 2019; Sampaio et al. 2019).

Este assunto faz-se de grande importância dado que a mudança no uso da terra reduziu uma boa porção do Cerrado a fragmentos de áreas nativas em uma matriz com grandes áreas degradadas (Lehmann & Parr, 2016; Borguetti et al. 2019). Mais de 50% do Cerrado já foi convertido, gerando um passivo ambiental de 5 milhões de hectares (MMA, 2017). A lei brasileira exige que este passivo seja restaurado (Lei nº 12.651/2012). O Cerrado é a savana tropical mais biodiversa do mundo e um grande hotspot, ameaçado e degradado principalmente pela expansão do agronegócio (Klink & Machado, 2005; Strassburg et al. 2017). O Bioma teve 30% da sua vegetação nativa retirada e substituída por pastagens para criação de gado, geralmente semeadas com GEI (Sano et al. 2019). A transposição de barreiras ecológicas possibilita que as GEI se tornem um fator de degradação, ocupando grandes áreas e competindo com espécies nativas (Silveira et al., 2018; Veldman et al., 2015). O governo brasileiro assumiu a responsabilidade de restauração em grandes áreas junto ao Bonn Challenge (2016). Para implementá-la, foi elaborado o Plano Nacional de Recuperação de Vegetação Nativa (PLANAVEG, 2017). Apesar da ampla demanda por restauração, ainda há uma série de gargalos como as fontes de financiamento, disponibilidade de sementes e o desenvolvimento de técnicas aplicáveis em larga escala, especialmente para os ecossistemas não-florestais, que predominam no Cerrado.

Além de ter uma grande diversidade de plantas e animais, o Cerrado desempenha papel importante para os ciclos do carbono e da água numa esfera que ultrapassa o Bioma (Bengtsson et al. 2019). Juntamente com os outros biomas abertos, detém cerca de 15% do carbono terrestre e 30% da produção primária, desempenhando um importante papel para o sequestro e estoque de carbono (Lal, 2004; Parr et al. 2014). No Cerrado encontram-se os principais cursos d'água tributários de três bacias hidrográficas que garantem o bem estar humano em terras nacionais e de outros países, como Argentina e Paraguai (Lima & Silva, 2007). Essa água é importante para a segurança energética da população, para a dessedentação de humanos e animais, para a produção agrícola, para a manutenção de serviços ecossistêmicos tanto no Cerrado como em outros biomas, como o Pantanal e o Chaco (Lima & Silva, 2007; Lima et al. 2011).

A restauração ecológica do Cerrado faz-se de extrema importância. O controle de GEI e a reintrodução de espécies nativas são necessárias para a restauração de ambientes de dossel aberto dominados por GEI no bioma. Neste estudo, pretende-se avaliar a eficiência do manejo integrado com uso de herbicida e da semeadura direta de espécies nativas para o controle de GEI e reintrodução de espécies nativas em ambientes abertos no Cerrado. O controle químico, associado a outras técnicas, têm o potencial de controlar as GEI e, consequentemente, tornar o local mais favorável para o estabelecimento de outras plantas (rebrota ou sementes). A semeadura direta é capaz de aumentar a quantidade de plantas nativas, aumentando também a riqueza e a cobertura destas, favorecendo o processo de restauração ecológica. Este trabalho está inserido num conjunto de esforços e experimentos de restauração ecológica no Parque Nacional da Chapada dos Veadeiros – Goiás e é pioneiro na utilização do manejo integrado de gramíneas exóticas e da semeadura direta de espécies nativas numa UCPI. Os resultados deste experimento agregam conhecimentos importantes para que grandes áreas degradadas sejam restauradas de forma mais eficiente e com menor custo, permitindo o cumprimento das metas firmadas internacionalmente.

Conforme o regulamento do Programa de Pós-Graduação em Ecologia da Universidade de Brasília, o artigo aqui apresentado com alterações, foi submetido para publicação na revista Scientific Reports, em inglês, e apresenta o detalhamento dos métodos, resultados e discussão do trabalho. Ao final, será apresentada uma conclusão geral do trabalho em português.

Objetivo

Este trabalho avaliou a eficácia do manejo integrado utilizando controle físico e aplicações de herbicidas combinados com a semeadura direta de espécies nativas para controle de gramíneas exóticas invasoras, bem como a reintrodução de espécies nativas em campos e savanas no Cerrado.

Hipóteses

A associação do controle químico com técnicas de controle físico permitirá o controle das gramíneas exóticas invasoras e o estabelecimento de outras plantas (por rebrota ou germinação de sementes). A semeadura direta aumentará a disponibilidade de propágulos de espécies nativas, aumentando a cobertura de espécies nativas e favorecendo a restauração ecológica.

Combination of techniques to control invasive grasses and restore Brazilian savannah - steps to achieve large-scale restoration

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Introduction

While crossing geographic and ecological barriers enables certain species to become invasive, biological invasions are considered an environmental degradation factor of global relevance [1-5]. Non-native grasses with invasive potential are distributed around the globe, generating well-documented impacts in the tropics [6;7]. In many cases, areas dominated by invasive grasses (IGS) are unable to return to their original state once abandoned, requiring active restoration with interventions aimed to accelerate restoration [8-11]. The control of IGS and the reintroduction of native species are among the most important practices to enable large-scale restoration [2;12-15]. Different IGS control methods have been tested [5;14;16]. Planting native trees in high densities has proven effective to shade out IGS [6;17;18]. In open ecosystems, however, this method is not recommended, as the native vegetation is light-dependent and characteristic groups of plants such as native grasses might be eliminated [4;15;19]. Therefore, the control of IGS is a challenge in the restoration of open ecosystems [14;20;21].

The use of fire (mechanical control) reduces IGS biomass and reduce the amount of recently-produced seeds, reducing spread and the presence of propagules, but is not effective to control IGS most widespread in pastures [21;22], since the majority of tropical grasses evolved with and are adapted to fire. The benefit between fire and IGS is often mutual, as fire-prone IGS alter fire regimes and produce more intense fires that further degrade the environment, increasing its susceptibility to wildfires and biological invasions [1;14;23;24]. Fire is therefore not effective on its own to control IGS [22;25]. Soil harrowing may eliminate IGS and expose seeds in the seed bank, favoring germination followed by destruction due to soil inversion by ploughing [21;26]. Control experiments have shown, however, that IGS are capable of recolonizing areas in process of restoration even when control is apparently successful in initial years [20;21;26-28].

Satisfactory results of IGS control in areas of open Neotropical Savannah (Cerrado) have been observed in integrated management projects that combine fire and/or harrowing with chemical control [6;26]. Chemical control of invasive non-native species using herbicides eliminates the barrier imposed by such species to the development of native species, facilitating regeneration at lower cost [6;13;29;30]. Integrated management herbicide application experiments in open Cerrado areas have led to the decrease of IGS cover and an increase in cover and richness of native species [26;31]. Even when IGS control is in place, the presence of even small amounts of seeds of native species against a high abundance of seeds of non-native species may hinder the recovery of the original ecosystems, therefore requiring the reintroduction of native species [2;32]. Direct seeding is an effective, low-cost technique to restore grasslands and savannahs that enable the simultaneous introduction of plants of different life forms [5;14;15;21;27;33].

Land use changes have reduced a large portion of the Brazilian Cerrado to fragments of natural areas in a matrix of vast degraded areas [34;35]. About 30% of the native vegetation in the biome has been replaced by pastures for cattle production, generally seeded with IGS [36]. The Cerrado is the most diverse tropical savannah in the world and a large biodiversity hotspot, threatened and degraded mainly due to the growth of agribusiness [37;38]. More than 50% of the Cerrado has been converted to other uses, creating environmental liability over 5 million hectares (MMA, 2017). Brazilian laws require these areas to be restored (Federal Law nº 12651/2012). The Brazilian Government has signed the Bonn Challenge (2016) and taken on responsibility for restoring large areas. The National Native Vegetation Restoration Plan (PLANAVEG) was developed in 2017 to guide the implementation of such efforts. Despite this large demand for restoration, several gaps remain, especially funding sources, seed availability, and the development of techniques for application to large areas, especially for non-forest ecosystems such as the Cerrado.

Apart from providing habitat for a high diversity of plants and animals, the Cerrado plays an important role for carbon and water cycles that extend beyond the biome [39]. The Cerrado and other open ecosystems stock about 15% of terrestrial carbon and support 30% of primary production [19;40]. The main tributaries of three water basins that provide water and ensure human well-being in Brazil and in other countries, namely Argentina and Paraguay, are located in the Cerrado [41]. These rivers are important for hydropower production, water provision to animals and humans, agricultural production, and the maintenance of ecosystem services in the Cerrado and other biomes, mainly the Pantanal and the Chaco [41;42].

Ecological restoration is of high relevance for the conservation of the Cerrado biome. The control of IGS and the reintroduction of native species are the main options for the restoration of open ecosystems, widely distributed in the biome. In this study, we evaluated the effectiveness of integrated management using herbicide applications combined with direct seeding of native species to control IGS, as well as the reintroduction of native species in open habitats. The association of chemical control with complementary restoration techniques has been proven to control IGS and, consequently, allow other plants to establish (by resprouting or seed germination). Studies show that direct seeding is able to simultaneously reintroduce plants of different forms of life, allowing them to establish themselves in the place, increasing the richness and coverage of native species, favoring ecological restoration.. The results of this experiment provide relevant knowledge for efficient large-scale restoration, facilitating the fulfillment of international goals and agreements.

Results

The data collected indicated herbicide spraying reduced the cover of IGS and direct seeding of native species influenced the plant communities after the first rainy season (3th sampling) in both experimental areas (Figures 1 (WdA) and 2 (WA)).

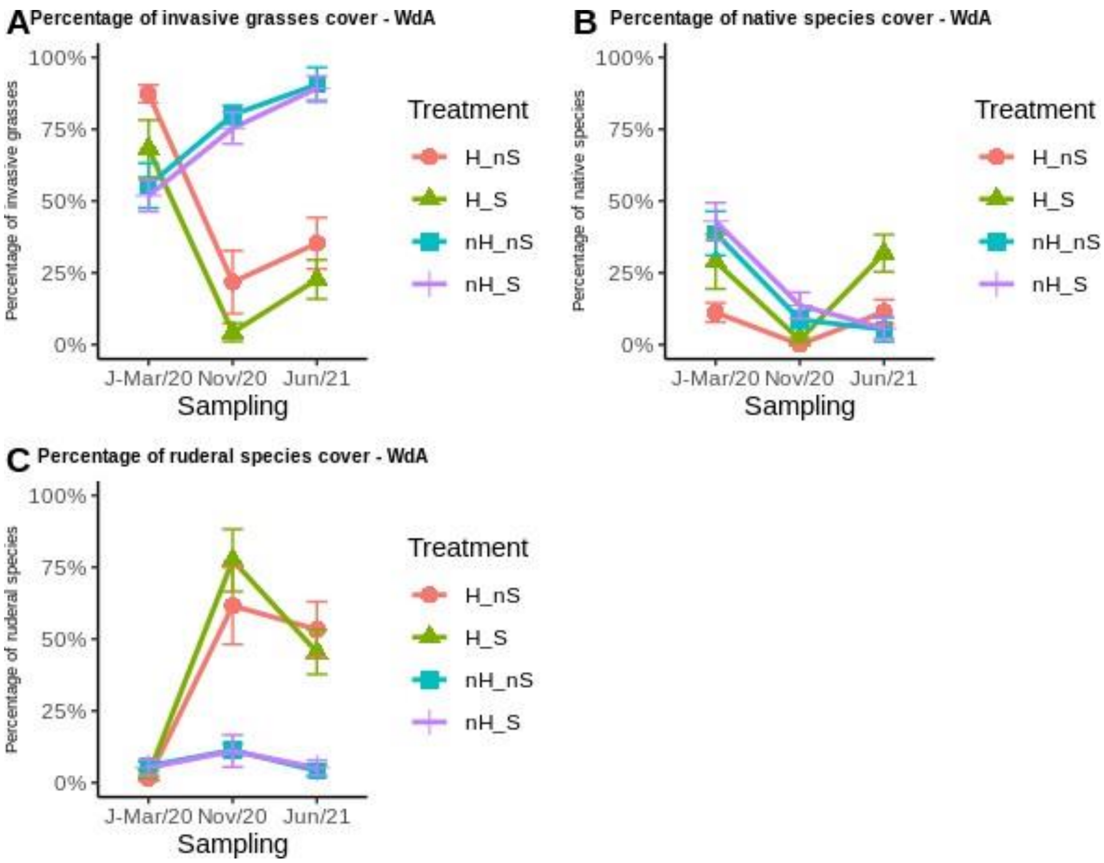


Figure 1: Line plots of plant cover of functional groups in each condition in the Well-drained Area (WdA) throughout the experiment. Sampling in January-March/2020 (J-Mar/20) was conducted after prescribed burning, harrowing, and herbicide application; in November (Nov/20), after herbicide application; and in

June/2021 (Jun/21) after direct seeding. (A) Relative IGS cover; (B) Native plant cover; and (C) Relative ruderal cover

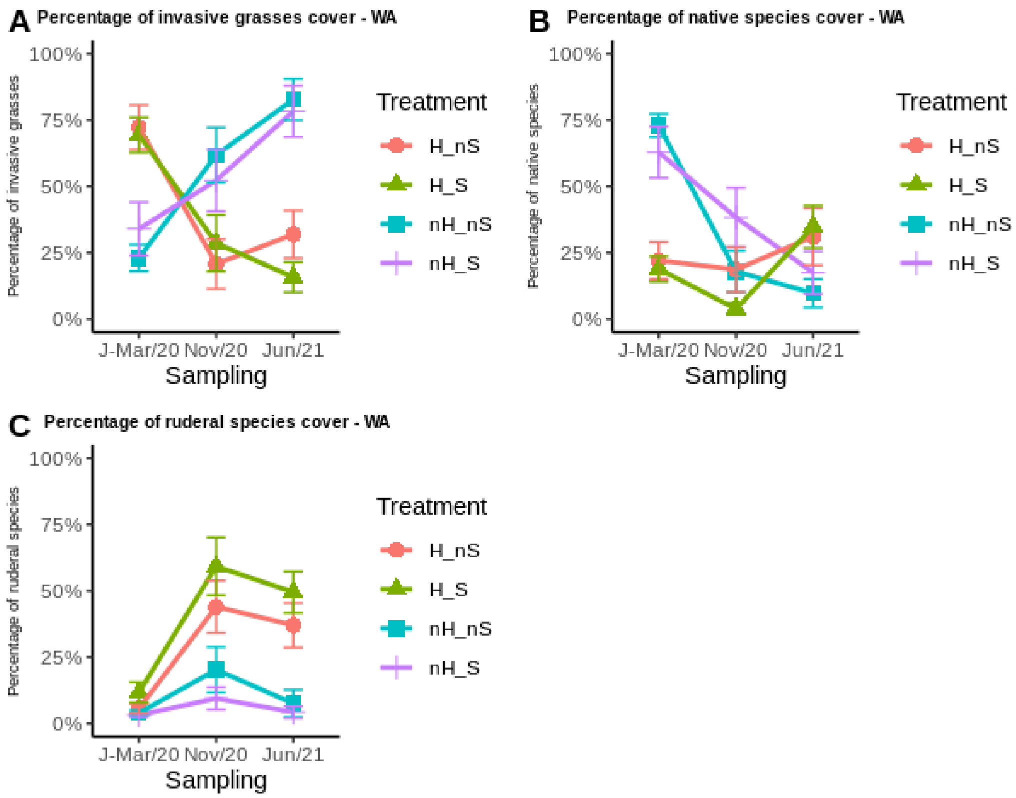


Figure 2: Line plots of plant cover of functional groups in each condition in the Waterlogged Area (WA) throughout the experiment. Sampling in January-March/2020 (J-Mar/20) was conducted after prescribed burning, harrowing, and herbicide application; in November (Nov/20), after herbicide application; and in June/2021 (Jun/21) after direct seeding. (A) Relative IGS cover; (B) Relative native species cover; and (C) Relative ruderal species cover.

The effects of herbicide application and direct seeding led to the development of plant communities with similar functional composition in both areas (Figure 3). Plant soil cover was highly reduced by herbicide application (Supplementary table ST1). Plots sprayed with herbicide resulted in lower plant cover values than non-treated plots (19.0 ± 12.1% and 70.5 ± 19.1% respectively for the well-drained area - WdA, and 34.7 ± 22.1% and 92.4 ± 10.4% for the water-logged area - WA). Herbicide spraying increased the amount of exposed soil, while the plots that were not sprayed remained mostly dominated by IGS.

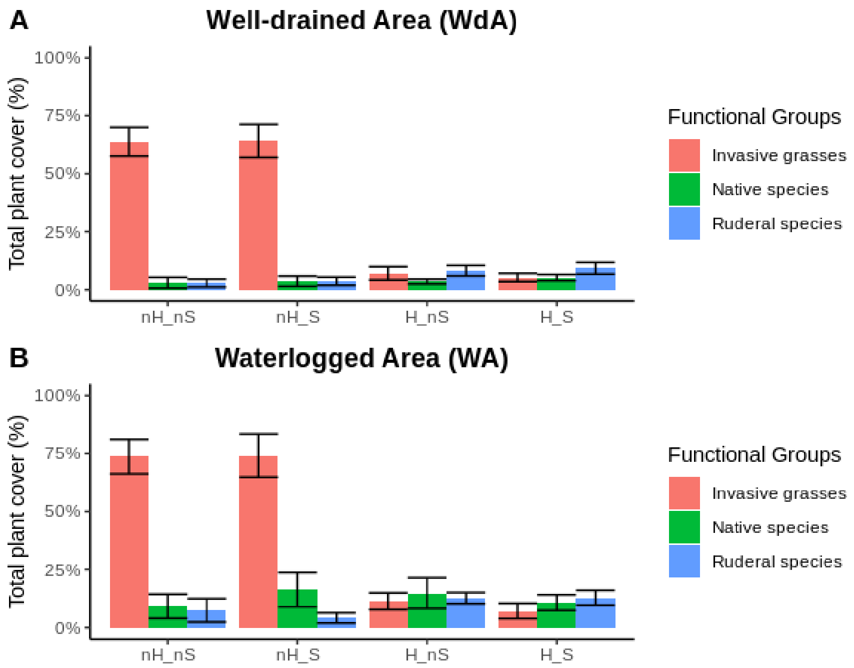


Figure 3: Total plant cover distributed in functional groups five months after seeding. Bars represent the cover means of each functional group, with standard error. Total plant cover not filled by combined % of functional groups is represented by exposed soil. Conditions - nH_nS: no herbicide, no seeding; nH_S: no herbicide, direct seeding,;H_nS: herbicide treatment, no seeding; H_S: herbicide treatment and direct seeding.

The Generalized Linear Models (GLMs) indicated that herbicide spraying was important in changing total plant cover as well as cover of functional groups in both areas (Supplementary table ST2). The models that best fit each variable indicated that herbicide application combined with direct seeding of native species affected total plant cover of IGS and native species in both areas, and of ruderal species in WA. The cover of ruderal species was the only one not directly affected by direct seeding in WdA.

At the end of the first rainy season after seeding, the plots treated with herbicide application in WdA had 28.9% (SD = ± 27.6%) average cover of IGS against 89.9% (SD = ± 17.5%) in plots where herbicide was not applied. Direct seeding only decreased IGS cover in the plots treated with herbicide (Figure 4A; Supplementary table ST1). The plots sprayed with herbicide resulted in higher cover of native species on average than the plots where herbicide was not sprayed (21.6 ± 21.2% and 5.4 ± 13.4%, respectively). Direct seeding significantly increased native species cover in plots sprayed with herbicide (Figure 4B). The control of IGS also benefited the development of ruderal plants, which reached 49.4% (±

30.0 %) cover on average against 4.6% (± 8.0%) in plots with no herbicide spraying applied (Figure 4C). Direct seeding did not affect the occurrence of ruderal plants in this area.

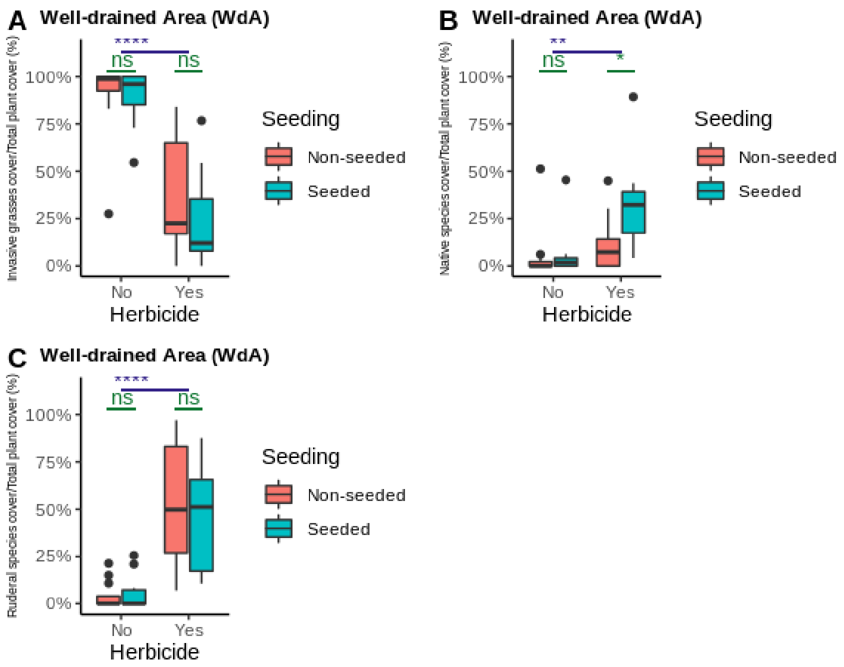


Figure 4: Plant cover by functional groups in each condition in the Well-drained Area (WdA) five months after seeding. Boxes represent quartiles and median, with standard errors and outliers of cover by functional group in each treatment combination. Horizontal bars indicate differences between pairs, asterisks indicate minimum significance with $p<0.05$, ns = non-significant. (A) Relative IGS cover; (B) Relative native species cover; and (C) Relative ruderal plant cover.

Herbicide spraying was also effective in WA, where the average IGS cover in non-treated plots was 80.5% (± 28.8%) against 23.8% (± 26.8%) in treated plots (Figure 5A). As observed in WdA, direct seeding benefited the results of chemical control by further contributing to reduce IGS cover (Supplementary table ST1.; Figure 5A). While herbicide application facilitated the development of native plants, direct seeding did not have significant effects in combination with herbicide spraying. Indigenous plant cover was 34.8% (± 27.8%) on average against 31% (± 37.3%) in plots without direct seeding (Supplementary table ST1; Figure 5B). Ruderal plants present in WA were also benefited by herbicide application, with 43.2% (± 28.1%) average cover in comparison with 5.9% (± 13.6%) on average in non-treated plots (Figure 5C). The combination of herbicide treatment and direct seeding resulted in higher cover by ruderal plants compared with herbicide treatment not followed by direct seeding (Supplementary table ST1).

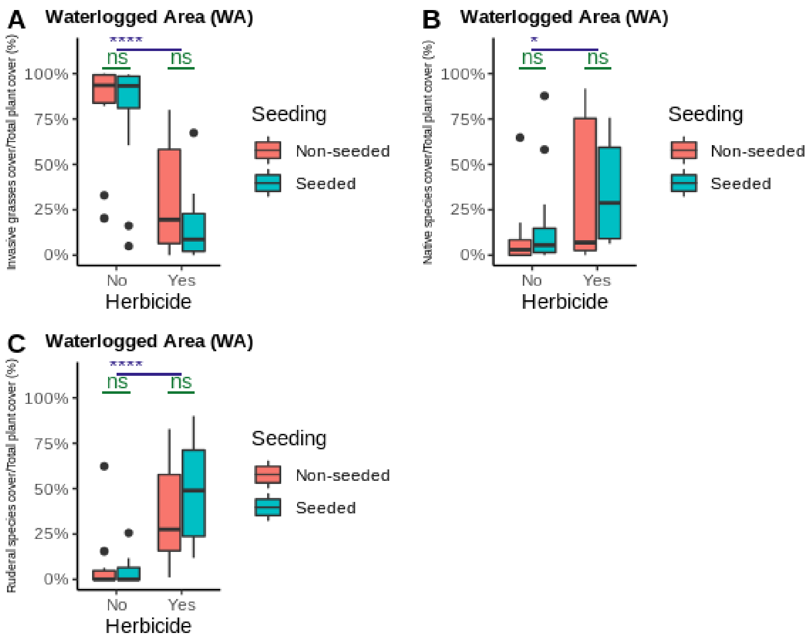


Figure 5: Plant cover of functional groups in the Waterlogged Area (WA) conditions five months after seeding. Boxes represent quartiles and median, with standard errors and outliers of cover by functional group in each treatment combination. Horizontal bars indicate differences between pairs, asterisks indicate minimum significance with $p<0.05$, ns = non-significant. (A) Relative IGS cover; (B) Relative native plant cover; and (C) Relative ruderal plant cover.

Discussion

Three herbicide applications following prescribed burning and three harrowing operations proved effective to reduce the abundance of IGS in the experimental plots. Direct seeding of native species was effective to increase soil cover by native plants in the well-drained area (WdA), but not in the seasonally waterlogged area (WA).

Herbicide spraying is used in ecological restoration projects around the world [2;6;9;44;45], and has also proven efficient in the restoration of open habitats in Cerrado. Positive restoration results using chemical control have been reported in Brazil for the Atlantic Forest [6;29;45;46], in subtropical grasslands in the south [13], in rupestrian formations in Cerrado [5], savannahs [26;31] and in grasslands in Central Brazil [47]. The benefits of herbicide application for ecological restoration have been corroborated by experimental and practical results in Brazil, and must be considered in projects that require control of IGS. In the experimental areas in our study, IGS cover was reduced, but the species have not been eradicated, as the number of interventions was not sufficient and the seed bank in the soil is still active. This creates risk to the successional process of plant communities, as

IGS are highly efficient in colonizing new areas, especially over exposed soils [20;28], as in the plots sprayed with herbicide.

Fast growth and biomass production of IGS create barriers for the development and establishment of native species [48]. The results obtained from our study show that IGS control increases the availability of resources at the local level, such as area of exposed soil, benefiting the growth of native and ruderal plants. Therefore, it is important to combine chemical control of dominating plants with direct seeding of native species, which then benefit from the absence of IGS [31;49]. Besides enabling the introduction of a wide range of species and functional groups of native species [27], direct seeding has potential to increase the cover of native plants in restoration projects. Given the colonization capacity of IGS, it is essential to conduct the regeneration of the plant community to cover the soil completely in order to avoid recolonization by IGS [20;28]. The choice of species is therefore of fundamental importance, and must include plants adapted to the restored habitat with high germination rates, establishment capacity, and growth. These facts and the lack of specific studies must be considered for understanding the results of the functional group of native species in WA, where seeding did not result in increased cover by native species even in the plots where IGS were chemically controlled.

Fast growth, tolerance to diverse environmental conditions, and intensive propagule production (seeds, stolons, and rhizomes) are traits that allow ruderal plants to quickly colonize fertile soils [50]. Experimental plots subjected to chemical control in both areas of our study were intensely colonized by ruderal species. This shows that ruderal plants may be competing with native plants developed from seeding, as well as with IGS. Nearby pasture areas may have facilitated the dispersal of ruderal plants able to colonize exposed soils [46]. Ruderal plants may hinder the establishment of native plants in initial stages of succession [46], but also seem to compete with IGS, creating obstacles to reinvasion. Over time, reintroduced native plants are able to outcompete ruderal plants in forest restoration projects (as well as IGS) due to the characteristics of introduced plants, mostly trees that grow higher than ruderals and shade them out [6]. In open ecosystems, however, this interaction will not exist, as no canopy will form over herbs or shrubs throughout the process of plant succession, allowing the effects of ruderal species to remain active over longer periods of time, which may benefit native plants or IGS. Although many ruderal species are native, while they cover the soil and compete with IGS, creating obstacles for reinvasion, they may also hinder the development of native plants reintroduced through seeding in order to conduct restoration to a more similar condition to the original habitat. Besides, in the mid and long-term, these species tend to disappear, not functioning as permanent allies for ecological restoration.

Low colonization efficiency by native species may be associated with factors such as seed dormancy, low germination rates, and species development time, among others. Combined with the presence of ruderal plants and IGS, these factors may pose problems for the fulfilment of international goals established by the Brazilian Government for restoring native vegetation in coming years (e.g. PLANAVEG and Bonn Challenge). In order to overcome this barrier, the production chain of native species seeds of high quality for ecological restoration needs to be strengthened. Experiments to improve species selection for the restoration of different habitats and vegetation types are urgent. There is also a gap of long-term studies to assess plant succession in restored areas of open ecosystems in the Neotropics, which curbs more affirmative conclusions on the effectiveness of the techniques applied over time. As much can be done to advance restoration in tropical open ecosystems, experiments must always consider the need for large-scale application.

Conclusions

The control of invasive grass species is a bottleneck in ecological restoration, especially in open ecosystems such as grasslands and savannahs. The control of the IGS made by integrated management combining prescribed fire and harrowing with chemical control allowed native and ruderal species to colonize the area. In the experimental areas treated with herbicide, the native plants introduced by direct seeding established successfully. Ruderal plants also established better in plots treated with herbicide, probably influencing the community trajectory, which may favor natives or IGS. Some challenges still need to be overcome in order to increase the effectiveness of long-term, large-scale restoration projects. We highlight the need to (1) assess whether new herbicide applications are required to avoid reinvasion by IGS; (2) develop more studies to gather data on phenological traits of species in different habitats, strengthening the seed production chain of native species and the effectiveness of restoration, and (3) develop long-term research to improve the understanding of the results of ecological restoration. To this moment, there is evidence that integrated management of IGS using fire, harrowing, and herbicide treatments is a promising way forward to achieve large-scale restoration. This approach will contribute to the fulfilment of ecological restoration goals in the Cerrado.

Methods

Study area

We conducted the experiment in an abandoned pasture in Chapada dos Veadeiros National Park, in the state of Goiás (14º07'03''S 47º38'30''W), Central Brazil. The region is characterized by AW climate in the Köppen classification, with hot, rainy summers, and cold, dry winters [51]. The average annual temperature varies from 20º to 27ºC and the average rainfall is 1617.8mm, concentrated between November and March. Altitude varies from 400 to 1676 meters in the park, which covers 2,406.11km² of forest, savannah and grassland physiognomies.

The original vegetation in the study area was open savannah with patches of wet grasslands in lower areas and "murundu grasslands" (grasslands punctuated with low hills covered by woody vegetation) in higher areas. The area was converted to pasture with non-native grasses in the 1980s, but had been abandoned for at least 20 years [20]. At the time, to provide a productive forage source for cattle, harrowing, liming, and fertilizationwere applied to the soil to reduce acidity [21]. These conditions favour the establishment of IGS [52]. Non-native grasses currently dominate the area, covering nearly 100% of the soil. *Urochloa decumbens* and *U. humidicola* are the dominant species, while *Andropogon gayanus* and *Melinis minutiflora* are also present [21].

Experimental design

We conducted the experiment in two different areas. The first one was originally savannah on well-drained soil, hereafter, well-drained area (WdA) (<20% clay), dominated mainly by *Urochloa decumbens* before the experiment was set up. The second area is seasonally waterlogged, called here waterlogged area (WA) (soil waterlogged three months of the year with >30% clay), originally a wet grassland, then dominated by *U. humidicola* before the experiment was set up. We subjected both areas to prescribed burning in March/2019 (Figure 6), which was carried out by professionals of the Fire Prevention and Control Brigade of the Chapada dos Veadeiros National Park. Harrowing was repeated three times with a tractor (Figure 6), during the dry season in 2019 as the initial effort to control non-native grasses, wear out the seed bank and relieve soil compaction [53;54]. We set up a complete factorial experiment in both areas, combining herbicide treatment (at two levels: treated and not treated) and direct seeding of native species (at two levels: with and without direct seeding). Which resulted in four possible combinations: herbicide treatment with direct seeding; herbicide treatment, with no seeding; no herbicide treatment, with direct seeding; and no herbicide, with no seeding. We established twelve 5 x 10 m plots for each combination in each area (WdA and WA), totalling 48 plots per area and 96 plots in total. The minimum distance between plots was 10 m.

We conducted the first plant cover sampling in the rainy season, between January and March, 2020, after prescribed burning and harrowing, and before herbicide treatment and direct seeding (Figure 6). We used the line intercept method to quantify the number of times each species touched the nine central meters of a measuring tape stretched diagonally across each plot [55]. We identified and classified the plant species in three functional groups based on plant origin: invasive grass species (IGS), native species (from direct seeding) and ruderal species (spontaneous). When it was not possible to classify plants in the field, we collected specimens and the respective functional groups were determined in the lab. The number of touches of each functional group was used to calculate the relative cover of each.

Glyphosate is the most common herbicide used in restoration projects to control grasses and it is licensed for use in Federal Protected Areas in Brazil [45]. We used a 3% glyphosate-based herbicide solution [46;57]. We applied the first spraying treatment ten months after burning and three months after the last intervention using the tractor (Figure 6). Two subsequent herbicide applications were conducted on IGS that recovered or germinated four and ten months after the first one, when grass tufts reached about 15 cm in height [47]. Both mechanical and chemical control were conducted by persons wearing personal protective equipment [6;29;46]. A 3m border around all plots was sprayed with herbicide on the same dates. In November/2020, at the end of the dry season, we conducted the second plant sampling using the same method as before, and registered the amount of exposed or covered soil in contact with the measuring tape to estimate cover.

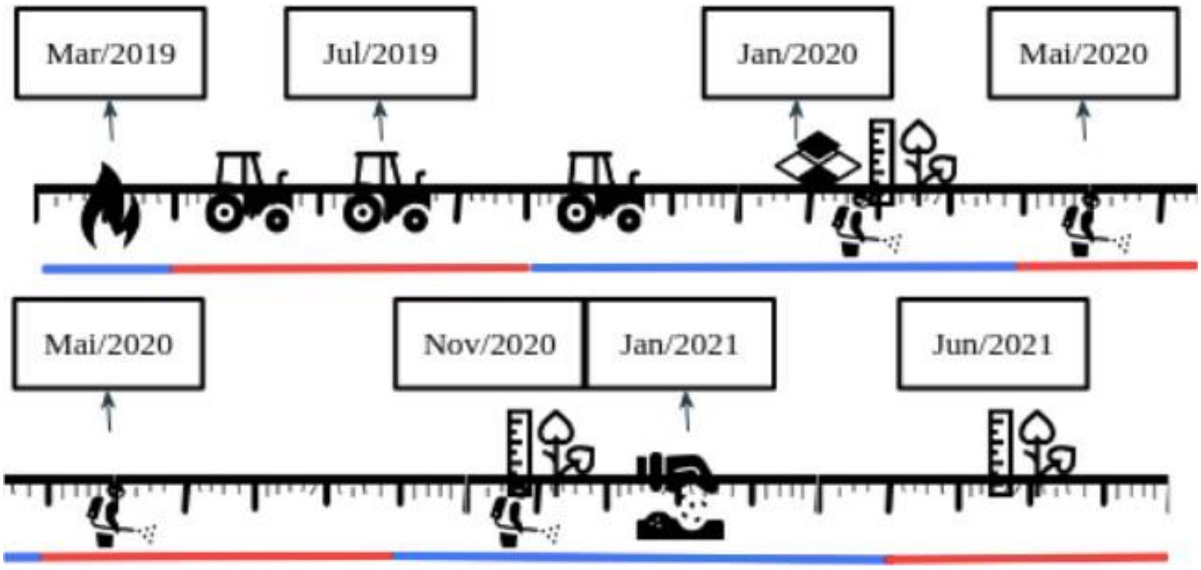
Direct seeding of native species took place in the rainy season, two months after the last herbicide treatment (Figure 6). Only graminoids, shrubs and subshrubs native to Cerrado grasslands and savannahs were used. We selected species from the Cerrado Seed Network (Rede de Sementes do Cerrado, RSC) for best adaptation to the environmental conditions of each area. Seeds were collected by professionals of the Associação Cerrado de Pé in the region where the experiment was carried out, taking advantage of plant adaptations to local environmental conditions, and strengthening the structure of native seed commerce for ecological restoration [58;59]. The average quantity of seeds of graminoids was 15.7kg/ha (10 spp.) and 12.5kg/ha of shrubs and subshrubs (3 spp.) in WdA and 22.74kg/ha of seeds of graminoids (16spp.) and 2.4kg/ha of shrubs and subshrubs (2spp.) in WA (Table 1). Despite the similarity in seed weight and higher richness in WA, there are less references or studies on the species used in this area than on the species used in the other area (WdA). Seed density per square meter can be highly variable between species (seed weight and size), varying between 5 – 1,100 viable seeds/m²/species [27]. In June, 2021 (dry season), we conducted the third plant sampling following the same method to verify plant cover by functional groups and soil cover. The sequence of events for implementing the experiment follows the timeline in Figure 6.

Table 1. Average seed weight of each species seeded per hectare (kilograms per hectare (10.000m²) and per plot (grams per experimental plot) in each area (Well drained Area (WdA) and Watterlogged Area (WA).

Well-drained Area				
Specie	Famíly	Growth Form	Kg/ha	g/plot
Lepidaploa aurea	Asteraceae	Shrubs	7.0	35.0
Vernonanthura polyantes	Asteraceae	Shrubs	3.4	17.0
Anacardium humile	Anacardiaceae	Shrubs	2.5	12.5
Andropogon fastigiatus	Poaceae	Graminoid	3.8	19.0
Aristida gibbosa	Poaceae	Graminoid	1.3	6.5
Loudetiopsis chrysothrix	Poaceae	Graminoid	2.0	10.0
Aristida flaccida	Poaceae	Graminoid	0.9	4.5
Axonopus barbigerus	Poaceae	Graminoid	0.6	3.0
Trachypogon spicatus	Poaceae	Graminoid	1.0	5.0
Hyparrhenia bracteata	Poaceae	Graminoid	0.4	2.0
Axonopus aures	Poaceae	Graminoid	0.6	3.0
Aristida riparia	Poaceae	Graminoid	2.3	11.5
Schizachtrium sanguineum	Poaceae	Graminoid	2.9	14.5
			Total = 28.7	Total = 143.5
Watterlogged Area				
Specie	Famíly	Growth Form	Kg/ha	g/plot
Achyrocline satureoides	Asteraceae	Subshrubs	0.7	3.5
Eremanthus uniflorus	Asteraceae	Shrubs	1.6	8.0
Andropogon fastigiatus	Poaceae	Graminoid	3.8	19.0
Loudetiopsis chrysothrix	Poaceae	Graminoid	1.9	9.5
Axonopus barbigerus	Poaceae	Graminoid	0.6	3.0
Watterlogged Area				

Specie	Famíly	Growth Form	Kg/ha	g/plot
Trachypogon spicatus	Poaceae	Graminoid	1.0	5.0
Hyparrhenia bracteata	Poaceae	Graminoid	0.4	2.0
Axonopus aures	Poaceae	Graminoid	0.7	3.5
Schizachtrium sanguineum	Poaceae	Graminoid	2.9	14.5
Rynchospora speciosa	Cyperaceae	Graminoid	0.5	2.5
Cyperaceae sp.	Cyperaceae	Graminoid	0.5	2.5
Andropogon bicornis	Poaceae	Graminoid	4.5	22.5
Andropogon leucosthachys	Poaceae	Graminoid	1.5	7.5
Paspalum sp.	Poaceae	Graminoid	0.7	3.5
Paepalanthus chiquitensis	Eriocaulaceae	Graminoid	1.4	7.0
Paepalanthus sp.	Eriocaulaceae	Graminoid	0.7	3.5
Xyris sp.	Xyridaceae	Graminoid	0.3	1.5
Schizachtrium sp.	Poaceae	Graminoid	0.6	3.0
			Total = 24.3	Total = 121.5

Figure 6. Timeline of the sequence followed for the implementation of the experiment. The fire icon is prescribed burn, tractor icons represent three harrowing, the plot icon is a set up of the plots, the man icon is the three applications of herbicide, and the plant/rule icon is the samplings. Each dash below the horizontal black line of the image represents a month. Colored line represent the rainy and dry season, with blue and red, respectively.



Data analysis

The relative cover of each functional group was calculated by dividing the number of times a functional group touched the line by the total number of touches in the plot. We built Generalized Linear Models (GLM) for each area using the glm function in the "stats" package [61]. We compared the relative cover values of IGS, native plants, and ruderal plants, and the proportions of plant cover of each functional group (response variables) with the effects of herbicide application and direct seeding of native species (fixed effect with two levels each). The models were run considering isolated fixed factors as well as the interaction between them for each of the response variables (binomial distribution) in each area. We built four models for each response variable and selected models using Akaike's Information Criteria [62]. Given that exposed soil encompasses the opposite area of plant cover, these values were interpreted only from the plant cover analysis. We used post-hoc tests (lsmeans function in "emmeans" package [63]) to compare the average values between the different combinations of the levels of each factor tested. We built the graphs using the "ggplot" package [64]. We conducted all analyses in R [61].

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Conclusão Geral

O controle de GEI é um gargalo para a restauração ecológica, principalmente de ambientes abertos como campos e savanas. Alguns desafios ainda precisam ser ultrapassados de modo a aumentar a efetividade de projetos de restauração de larga escala a longo prazo. Neste estudo, o controle de GEI não foi totalmente eficaz, mantendo a competição destas com as nativas e a possibilidade de uma reinvasão. A reavaliação dos intervalos entre as aplicações do herbicida pode levar a um controle mais eficaz das GEI que ainda persistem no local. Considerando que a aspersão do herbicida é feita de forma pontual nas GEI, aspersões pós semeadura de nativas podem ser necessárias para se evitar uma reinvasão e um melhor desenvolvimento das nativas presentes no local. Um maior número de gradagens pode também contribuir para um melhor controle inicial das espécies invasoras.

São necessários mais estudos para obtenção de informações sobre as características fenológicas de espécies presentes nos diversos ambientes. As espécies ruderais que cobrirm boa parte do solo nas duas áreas podem apresentar diferentes efeitos em cada comunidade, com possíveis efeitos negativos de longo prazo. O risco de uma nova invasão por alguma outra espécie ou grupo funcional pode levar a uma menor diversidade no local, impedindo também o desenvolvimento de plantas nativas. Mais informações fenológicas de diferentes espécies nativas podem favorecer decisões sobre espécies e quantidades de sementes a serem utilizadas nos diversos ambientes. Fortalecendo assim a cadeia produtiva de sementes nativas e a efetividade da restauração. Essas informações podem ser adquiridas com mais pesquisas de longo prazo, melhorando o entendimento dos resultados da restauração ecológica.

Os resultados do experimento aqui apresentado são ainda preliminares, mas com grande importância ecológica e legal. Até o momento, os resultados indicam que o controle integrado de GEI com o uso do fogo, gradagem e herbicida é um caminho promissor para um melhor controle de GEI em projetos de restauração em larga escala. Bons resultados do uso do controle químico em uma UCPI pode abrir portas para a visibilidade de uma técnica melhor para o controle de GEI nos ambientes abertos no Cerrado, mostrando que a utilização correta do herbicida pode ser benéfica, no longo prazo, para a restauração ecológica de um ambiente invadido por GEI. Juntamente com a nova técnica de controle de GEI, a semeadura direta de sementes nativas faz-se de extrema importância para a reintrodução de diversas espécies nativas onde estas já não estão mais presentes. Assim, os resultados da utilização do controle integrado de GEI com fogo, gradagens e herbicida, e a reintrodução de nativas através da semeadura direta contribuem para que as metas de restauração ecológica para o Cerrado sejam alcançadas pelo poder público e privado.

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Material suplementar

Table ST1: Contrasts between conditions for relative plant cover of each functional group and total plant cover in the experimental areas (well drained area (WdA) and waterlogged area (WA).

	estimate	SE	df	z.ratio	p.value
Invasive grasses at well drained area					
NH NS - NH S	-0.207434613999249	0.16991981571825	Inf	-1.22077941953046	0.222169549081193

Fixed effects	AIC	ΔAIC	Pseudo-R²
Models for invasive grasses at well drained area			
~Herbicide	914.2156	39.2856	0.582
~Sowing	1874.68	999.75	0.014
~Herbicide+Sowing	899.22	24.29	0.602
~Herbicide+Sowing+Herbicide:Sowing	**874.93**	**0.0**	**0.602**
Models for natives at well drained area			
~Herbicide	629.4404	47.9659	0.271
~Sowing	799.9626	218.4881	0,078
~Herbicide+Sowing	612.1123	30.6378	0.352
~Herbicide+Sowing+Herbicide:Sowing	**581.4745**	**0.0**	**0.372**
Models for ruderal at well drained area			
~Herbicide	**657.8801**	**0.0**	**0.550**
~Sowing	1280.22	622.3399	0.001
~Herbicide+Sowing	659.7076	1.8275	0.554
~Herbicide+Sowing+Herbicide:Sowing	661.6286	3.7485	0.555
Models for vegetal cover at well drained area			
~Herbicide	6536.331	496.973	0.684
~Sowing	18349.32	12309.96	0.027
~Herbicide+Sowing	6042.9	3.542	0.720
~Herbicide+Sowing+Herbicide:Sowing	**6039.358**	**0.0**	**0.721**
Models for invasive grasses at waterlogged area			
~Herbicide	1733.62	2.668	0.486
~Sowing	2745.586	1014.634	0.015
~Herbicide+Sowing	**1730.952**	**0.0**	**0.498**
~Herbicide+Sowing+Herbicide:Sowing	1732.157	1.205	0.500
Models for natives at waterlogged area			
~Herbicide	1696.322	20.265	0.138
~Sowing	1991.858	315.801	0.048
~Herbicide+Sowing	1695.317	19.26	0.171
~Herbicide+Sowing+Herbicide:Sowing	**1676.057**	**0.0**	**0.171**
Models for ruderal at waterlogged area			
~Herbicide	854.4744	35.3653	0.527
~Sowing	1317.35	498.2409	0.004
~Herbicide+Sowing	856.1772	37.0681	0.533
~Herbicide+Sowing+Herbicide:Sowing	**819.1091**	**0.0**	**0.540**
Models for vegetal cover at waterlogged area			
~Herbicide	7506.364	295.356	0.648
~Sowing	24625.05	17414.04	0.001
~Herbicide+Sowing	7482.547	271.539	0.651
~Herbicide+Sowing+Herbicide:Sowing	**7211.008**	**0.0**	**0.655**

Table ST2: Generalized linear models (GLM) for relative plant cover of each functional group and for total plant cover in the experimental areas (well drained area (WdA) and waterlogged area (WA). The model that best fit the response variable evaluated is in bold.