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Management techniques for the control of *Melinis minutiflora* P. Beauv. (molasses grass): ten years of research on an invasive grass species in the Brazilian Cerrado

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ABSTRACT

The invasion of exotic species is considered to be a major threat to the preservation of biodiversity. In the Parque Nacional de Brasília (National Park of Brasília), the invasive *Melinis minutiflora* (molasses grass) occupies more than 10 % of the area of the park. The present, long-term, study compared two treatments of exposure to molasses grass: 1) fire and 2) integrated management (fire + herbicide sprays + manual removal). The aerial biomass of molasses grass in the experimental area initially represented ca. 55 % of the total aerial biomass, a percentage that apparently did not influence native plant species richness at this site. Fire alone was not sufficient to control molasses grass, which attained its pre-treatment biomass values after two years. Integrated management reduced, and maintained, biomass to less than 1 % of its original value after ten years, and maintained this level throughout the study, demonstrating that it is a promising strategy for the recovery of areas invaded by molasses grass in the Cerrado. However, because of the recolonization by molasses grass, long-term monitoring efforts are targeting outbreaks, which would require immediate intervention in order to maintain the native biological diversity of the region.

Keywords: fire, glyphosate, ground layer biomass and species richness, protected areas, recolonization

Introduction

The Cerrado consists of a wide variety of savanna landscapes that dominate central Brazil occupying approximately 2.0 million km^2. Its complex and diverse flora harbors a vast genetic heritage and is home to numerous endemic species (Klink & Machado 2005). According to Mendonça *et al*. (2008), the Cerrado has 11627 native species, including ferns (385), gymnosperms (4) and angiosperms (11238), which are distributed in 1,521 genera and 193 families. Forty-four percent of its flora is endemic, and in that sense it is considered the most richenes tropical savanna in the world (Klink & Machado 2005). Despite its status as one of world biodiversity hotspots (Myers *et al*. 2000; Silva & Baetas 2002) in the last 35 years more than half of its original vegetation has been transformed into pastures and annual crops (Consorte-McCrea *et al*. 2013) and is being threatened by biological invasions ((Filgueiras, 1990; Pivello *et al*. 1999; Ribeiro *et al*. 2017).

The process of biological invasion is a global phenomenon and its impact is evident in different ecosystems (D'Antonio & Vitousek 1992; Turner *et al*. 2008; Rossiter-Rachor *et al*. 2009; Gardener *et al*. 2013). Recent meta-analyses of field studies (Vilà *et al*. 2011; Scasta *et al*. 2015) provide

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convincing evidence that invasive plant species exert significant impacts on many ecological variables. However, the magnitude and direction of these impacts vary among different levels of ecological complexity. Among the major invasive species, grasses stand out for their great invasive potential, with many possessing characteristics common to key invasive plant species, such as a high capacity for sexual and asexual reproduction, production of large quantities of easily dispersed seeds, high germination and growth rates, high photosynthetic efficiency and nutrient use, and tolerance to herbivory (Baruch *et al*. 1985; D'Antonio & Vitousek 1992; Williams & Baruch 2000; Rossiter-Rachor *et al.* 2009; Martins *et al*. 2011).

Several grass species native from Africa and Asia have been introduced, either accidentally or for commercial purposes, into different countries and have spread over large extensions of natural ecosystems and due to their high competitive advantage have displaced native species, (Parsons 1992; Filgueiras 2005; Coleman & Levine 2007; Rossiter-Rachor *et al*. 2009). The African grass *Melinis minutiflora* (molasses grass) was probably introduced into Brazil in the 18th century during intense slave trade in the colonial period and is now considered an invasive species (Filgueiras 1990). For nearly 120 years *M. minutiflora* and *Hyparrhenia rufa* (jaragua grass) were used as the principal forage grass species in Central Brazil and in the 1960s approximately 30 of the 123 million ha of Brazil's pastures were planted with *M. minutiflora* (Joviano & Costa 1965; Teixeira 1984). Because of its low biomass production and low capacity to withstand intensive trampling, a program for its replacement by more productive species, such as *Andropogon gayanus*, *Urochloa decumbens*, and *Urochloa brizantha* was initiated in the 1970s (Boddey *et al*. 2004). Even though it is no longer used as a forage grass, *M. minutiflora* persists in Central Brazil because of its adaptability to soils and climatic conditions in this region (Curado & Costa 1980; Botrel *et al*. 1988; Martins *et al*. 2004).

The management plan of the PARNA Brasília (National Park of Brasília), based on the study Vegetation and Flora of PARNA Brasília (Funatura/Ibama 1998), considers *M. minutiflora*, due to its aggressiveness, to be responsible for the greatest impact on the native flora of this protected area. This grass species was present in the PARNA Brasília even before its creation in the early 1960s and it is currently present in about 4,500 ha of the Park (Martins 2006). The first record of the invasion by *M. minutiflora* in Brazil is from the early 1950s when it was reported to have spontaneously spread to areas next to planted pastures occupying vast areas without human intervention (MA 1953). Due to its high biomass accumulation, its large production of viable seeds, with high germination rates and rapid growth, *M. minutiflora* is considered to be a real threat to the conservation of the savanna grasslands in this protected area (Funatura/Ibama 1998; Martins *et al*. 2009; Carmona & Martins 2010; Martins *et al*. 2011).

The development of management treatments for the control of invasive alien species is a major challenge for managers of protected areas (Mason & French 2007; Foxcroft & Downey 2008; Downey 2010; Horowitz *et al*. 2013). Physical, chemical and biological control are the principal methods (Bossard *et al*. 2000). All methods have advantages and disadvantages and, according to the previously cited authors, the use of several methods in conjunction is recommended. Published results on the use of integrated management treatments indicate that Brazil has contributed to this line of research, especially regarding preventive measures (Harker & O'Donovan 2013), but few studies in Brazil have focused on *M. minutiflora*. In this paper we compare results of our experience in applying different management treatments for the control of *M. minutiflora* over a 10 year period in an area of Cerrado.

Materials and methods

Study area and experimental site

The study was conducted in the PARNA Brasília, created by Decree No. 241/61 (30,000 ha), and located between 15º34' and 15º45'S and 48º05' and 48º53'W. Law No. 11.282, of March 8th, 2006, redefined its polygonal area and added another 11,000 ha to its northwestern limits. The altitude of the PARNA Brasília varies from 1,070 to 1,200 m. Its climate is tropical (Koppen Aw), with an average annual rainfall of about 1,600 mm and more than 90 % is concentrated from November to April. The phanerogamic flora of PARNA Brasília is only partially known and the available data include 1,223 species, distributed in 539 genera and 104 families. The best represented families are Poaceae (158 spp), Compositae (129 spp), Leguminosae (118 spp), Orchidaceae (82 spp), and Myrtaceae (52 spp) (Filgueiras 1991; Funatura/Ibama 1998; Proença *et al*. 2001; Unesco 2002).

The experimental area with a size of 2,400 m^2 (40 x 60 m) is located within the Special Use Zone, next to the Intensive Use Zone of the PARNA Brasília, approximately 400 m from the Park headquarters, at 15º43'53"S and 47º55'35"W. Its vegetation is classified as "cerrado ralo", savanna vegetation with sparsely spaced trees (Ribeiro & Walter 1998) and according to Coelho (2002) the last fire in the area was in 1989. The exact date of introduction of *Melinis minutiflora* P. Beauv. to the site where this experiment was conducted is not known, however this area was used in the 1970s and 1980s as a pasture for the horses that were used to patrol the National Park and probably dispersed seeds of this species within the area.

Target species

Melinis minutiflora has a wide geographic distribution in predominantly tropical and subtropical regions, between 30º north and south of the equator. Eastern Africa is considered to be the center of origin of *M. minutiflora* (Mitidieri 1983), with Brazil considered its center of development and dispersion (Vello 1975). It is an aromatic perennial herbaceous species with C_4 photosynthetic metabolism, with an average height between 0.40 and 0.60 m, but can attain a height of 1.6 m or more. Its inflorescences are terminal, narrow panicles, 10 to 30 cm long, purplish, with short branches. Its seeds are quite small, 1.5 to 2.5 mm long and its caryopsis is 1.2 to 1.4 mm long (Mitidieri 1983; Martins 2006). The species produces large quantities of seeds from 13 to 15 million seeds/kg (200-280 kg/ha). It is well adapted to tropical and sub-tropical climates with annual rainfall above 1,200 mm (Mitidieri 1983; Skerman & Rivers 1992; Martins 2006). It is considered to be quite palatable to cattle, but compared to other forage grasses its productivity is low, between 3 and 11 tons of dry matter per year (Bogdan 1977; Mitidieri 1983; Skerman & Rivers 1992). Between 1947 and 2012, 127 articles have been published on *M. minutiflora* in Brazil, including studies on management to biological invasion (Rodovalho & Nardoto 2014).

Management treatments for the control of *M. minutiflora*

The management treatments used in this study were: burn/May (BM), burn/September (BS), integrated management/May (IMM), and integrated management/September (IMS). Control plots, with no interventions either in May (CM) or September (CS) were also included in the experimental design. The first management technique - burning - refers to controlled burns either in May of 2003 (BM), before the flowering of *M. minutiflora*; or in September of 2003 (BS), after its flowering. The second technique - integrated management - consists of three different interventions: 1) controlled burns either in May of 2003 or in September of 2003, after its flowering; 2) spot application of a herbicide in January of 2004 in both the IMM and IMS plots. The herbicide application was limited to clumps that re-sprouted after the controlled burn and another spot application in April of the same year, limited to established seedlings, again in both IMM and IMS plots; and 3) manual removal of seedlings of *M. minutiflora* in January, February, and March of 2005 in the IMM and IMS plots. From 2006 to 2013, the manual removal of resprouts or seedlings in the IMM and IMS plots was always conducted in April. All controlled burns were conducted from 14:30 to 16:00, on May 5 (BM only) and May 6 (IMM only) of 2003, and on September 2 (BS only) and September 3 (IMS only) of 2003. Since data on rainfall area not collected in the PARNA Brasília, we do not know the extension of the dry spells prior to each controlled fire. Due to the time necessary to make firebreaks between experimental plots, the burns were done on consecutive days to have similar initial climatic conditions for each seasonal treatment. Control plots had no interventions in either May or September. Four replicates of each treatment were made within the experimental area and the dimensions of each treatment and control plots were 100 m^2 (10 x 10 m), with borders of 0.5 m (Fig. 1). In all the burns, the fire front was pushed by wind.

We recorded the following environmental parameters in the vicinity of the study area immediately prior to the controlled fires in May and September of 2003: air temperature (°C), relative humidity (%), and wind speed (m/s). The herbicide used in all chemical treatment interventions was RoundupÒ or glyphosate (N-(phosphonomethyl) glycine), at a concentration of 0.5 % v/v, as recommended in the instructions for use of this product, was used in all chemical treatment interventions.

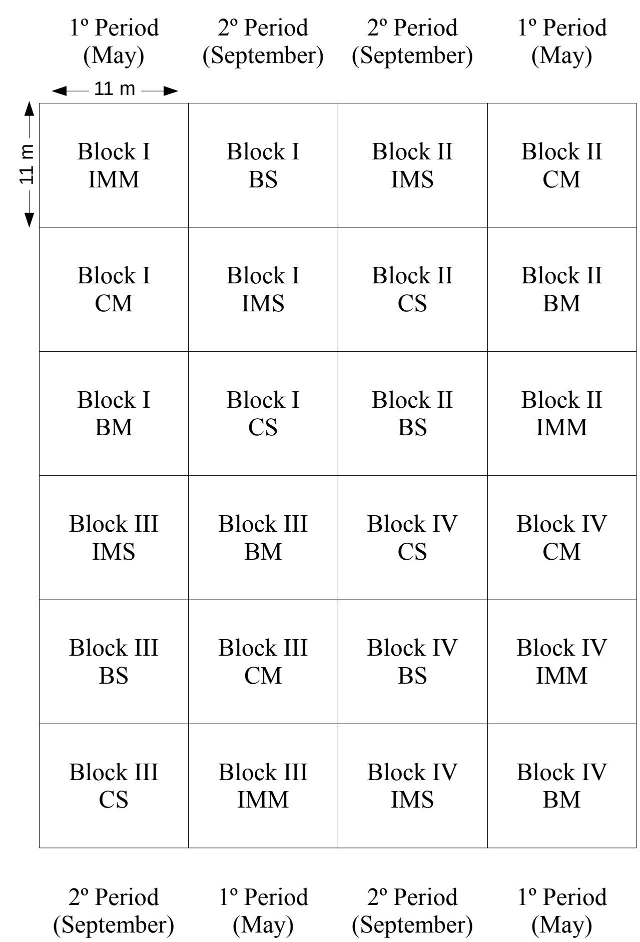

Figure 1. Croqui of the experimental plot. The header 1st Period refers to treatments started in May and the header 2nd Period refers to treatments started in September in the PARNA Brasília, Brasília, DF, Brazil. Each group of six plots (separated by thicker black lines) includes one plot of each treatment used. These were: control May (CM), control September (CS), burn/May (BM), burn/September (BS), integrated management/May (IMM), and integrated management/September (IMS).

The initial concentration of the glyphosate was 480 g of soluble concentrate per liter. A costal jet PJH sprayer, equipped with full cone spray nozzles TG-2, with a flow rate of 1.22 liters per minute, was used for application of glyphosate, with an estimated consumption of 1,220 liters of herbicide spray per ha. In the second application, when only by a few individuals of *M. minutiflora* were present, herbicide consumption was very low, about 0.8 liters of glyphosate per ha. The use of glyphosate within the limits of the National Park was approved by the Chico Mendes Institute for the Preservation of Biodiversity (ICMBio) the agency responsible for management of Federal conservation units in Brazil.

Biomass of the herbaceous and subshrub

To evaluate the recovery of the ground layer we collected aboveground biomass in the following periods: April 2003 before the management application, April 2005, April 2008, and April 2012. On each collection date ten quadrats of 0.25 m^2 (0.50 x 0.50 m) were randomly located in each replicate of each treatment and all aerial biomass of the ground layer, live and dead, was collected. Subsequently, the collected material was taken to the Ecology Laboratory of the University of Brasília for separation into two components: 1) total biomass = sum of native grasses + dicotyledons with stem circumference of < 1 cm, and 2) biomass of *M. minutiflora*. The material was then oven dried at 70 °C for 48 hours to determine its dry weight.

Species richness

Using the method of Filgueiras *et al*. (1994) we recorded all species present in all plots beginning in October of 2002. These evaluations were performed weekly for the first two years and subsequently monthly until May of 2012. All species in each plot were identified and any unknown species was collected. Identification of the unknown species was made by consulting specialists or by comparison with dried specimens in the Herbarium of the University of Brasília (UB) or CENARGEN (CEN). All vouchers were deposited in the Herbarium of the University of Brasília. The Sørensen similarity index (Gomide *et al*. 2006) was used to compare floristic similarity. Among treatment floristic similarity was calculated from presence/absence data using the entire species list from all 10 years of collection for each plot. The data from plots within the same treatment type were pooled prior to the analysis.

Statistical analyses

We used a randomized block design (RBD) with split plots to compare treatments for evaluation of the total biomass (all native species + *Melinis*) and that of *M. minutiflora*. The two factors in the analysis were: 1) "period" for the plot condition and 2) "management" for the subplots. "Period" refers to months: May (before flowering) and September (after seed dispersal). "Management" consisted of: control (CM and CS), controlled burns (BM and BS), and integrated management (IMM and IMS). Statistical verification of the significance of the treatments was done using a two-way Analysis of Variance (ANOVA). The Tukey Test (Honestly Significant Difference, HSD), at the probability level of 5 %, was used for comparison of means. When a variable did not fulfill the pre-requisites of normality and homogeneity of variance the Friedman test was used since this is a non-parametric alternative for experiments using random blocks. All analyses were performed using the software Statistix 8 (Analytical Software 2003) and SAS Version 9.1.2 (2004).

Results

Management treatments for the control of M. minutiflora

Approximately 97 % of the combustible material was consumed in the BM and the IMM plots during the controlled burns. In the BS and the IMS plots virtually all of the available combustible material in both treatments was consumed in the controlled burns. The values of the percentage of combustible material consumed were visually estimated in the plots. Although no climatic data were collected prior to either controlled burn in May or September, the climatic conditions on the days of each burn (Tab. 1) were qualitatively comparable for the time of year when the plots were burned.

The spraying of glyphosate was done on January 15, 2004, between 8:30 and 11:30 am, and on April 30, 2004, between 2:00 and 4:30 pm. A concentration of 0.8 l of glyphosate per ha was used in all applications. The first visual symptoms of the herbicide effect were observed between 7 and 15 DAT (days after treatment), starting with chlorosis, which accentuated over time, leading to plants prostrating, yellowing, and drying.

Table 1. Climatic and biomass characteristics of the applied treatments: burn/May (BM), burn/September (BS), integrated management/May (IMM), and integrated management/September (IMS) in the National Park of Brasília.

Burn regime	Temperature (°C)	Relative Humidity (%)	Wind speed (m/s)	Biomass* (t/ha)
BM	29.4	44.2	0.8	8.9
IMM	28.6	48.5	1.1	8.0
BS	31.9	29.2	1.7	8.4
IMS	30.6	30.7	1.3	6.7

*Herbaceous aerial biomass (ground layer) included grasses, herbs, and woody species that had stems of at least 1cm in diameter and the height similar to that of the grass layer.

Biomass of the herbaceous and subshrub stratum

Prior to the treatments performed in 2003, there were no significant difference in values of the total biomass (sum of native species and *M. minutiflora*) or in the biomass of *M. minutiflora* in the control plots and the plots reserved for the evaluated treatments (Tabs. 2, 3). These data indicate that initially *M. minutiflora* had a fairly homogeneous distribution throughout the experimental area.

The only significant difference in the total biomass in the collections subsequent to the application of different management treatments (2005, 2008, and 2012) was found in 2005. In May of 2005, the control plot values had statistically higher values (Tab. 2) than those in the integrated management treatment ($F_{14,9}$ = 4,52; p = 0,01). The same result was observed in September of 2005. No significant difference in total biomass was observed between collection dates in any comparison. No statistical difference due to interactions were found.

For biomass of *M. minutiflora* in all three collections in 2005, 2008 and 2013 and independent of the month of the initial treatment (May or September in 2003), the results (Tab. 3) showed that the control plots (CM and CS) and plots subjected to controlled burns (BM and BS) had similar values but the Integrated Management plots (IMM and IMS) were statistically different ($\chi^2 \cong 7.5$, $p \cong 0.02$ in all comparisons) reducing the biomass in 99 % compared to the initial values. No significant difference in biomass of *M. minutiflora* was observed between collection dates in any comparison.

Species richness in the experimental area

A total of 432 species distributed in 232 genera and 64 families were recorded for the experimental area (0.24 ha) during the 10 years. There was an increase in species richness compared to data from the first three years after the start of this experiment, when 402 species in 213 genera and 60 families were reported (Martins et al 2011). The most representative families, in both studies were Asteraceae (68 to 75 species), Poaceae (53 to 57 species), Fabaceae (50 to 54 species), Malpighiaceae (20 to 21 species), Myrtaceae (18 to 19 species), and Lamiaceae (15 species). These families represented 55 % of the total collected species. Over the ten year period in this study the following species richness numbers were recorded: CM (276 species in 400 m^2); CS (260 species in 400 m^2); BM (282 species in 400 m^2); BS (299 species in 400 m^2); IMM (287 species in 400 m^2); IMS (329 species in 400 m^2). According to the Sørensen similarity index, the areas were quite similar in their floristic similarity (Is = 0.76 to 0.80) (Tab. 4). A manuscript with a complete list of all species present in the plots is in preparation.

Discussion

Management treatments for the control of *M. minutiflora*

In many parts of the world controlled burns have been used as a management tool to control invasive alien species (DiTomaso *et al*. 2006; Chaudhari *et al*. 2012; Ruckman

Table 2. Average and standard deviance total biomass distribution (t/ha) per evaluation periods and management treatments in the National Park of Brasília.

Management treatments	2003		2005		2008		2013	
	May	September	May	September	May	September	May	September
Control	6.31±1.47Aa	6.80±0.60Aa	6.85±1.30Aa	6.65±0.90Aa	7.30±1.69Aa	7.19±1.74Aa	6.02±1.25Aa	5.15±0.96Aa
Burn	6.63±0.97Aa	7.50±1.87Aa	5.63±1.17ABa	4.49±1.10Ba	6.53±0.29Aa	7.02±1.69Aa	5.90±0.48Aa	5.23±0.56Aa
Integrated management	7.42±0.41Aa	6.48±0.95Aa	3.76±0.73Ba	3.48±0.62Ba	5.22±1.08Aa	5.04±0.67Aa	4.63±0.13Aa	5.17±0.55Aa
Total	20.36	20.78	16.25	14.63	19.06	19.19	16.56	15.57

Capital and lower-case letters indicate significant differences in management (columns) and per period (rows within years), respectively. The Tukey test, at 5 % probability.

Table 3. Average and standard deviance biomass distribution (t/ha) of *Melinis minutiflora* per evaluation periods and management treatments in the National Park of Brasília.

Management treatments	2003		2005		2008		2013	
	May	September	May	September	May	September	May	September
Control	3.39±1.37Aa	2.93±2.34Aa	2.53±1.59Aa	2.91±2.56Aa	2.60±2.80Aa	2.48±3.13Aa	2.73±2.26Aa	1.87±0.66Aa
Burn	4.02±1.50Aa	5.12±2.13Aa	1.66±1.24Aa	0.96±0.41Aa	1.72±1.06Aa	2.04±2.06Aa	1.72±0.82Aa	2.45±2.35Aa
Integrated management	3.84±1.20Aa	3.99±0.79Aa	0.021±0.016Ba	0.020±0.018Ba	0.016±0.009Ba	0.018±0.006Ba	0.007±0.004Ba	0.007±0.003Ba
Total	11.26	12.05	4.21	3.01	4.34	4.54	4.46	4.33

Capital and lower-case letters indicate significant differences in management and per period, respectively. The Tukey test, at 5 % probability.
*In these cases it was applied the non-parametric Friedman´s test, at 5 % probability.

Table 4. Sørensen similarity index for the applied treatments: CM (control/May), CS (control/September), BM (burn/May), BS (burn/September), IMM (integrated management/May), and IMS (integrated management/September) in the National Park of Brasilia.

Treatments	CM	CM	BM	BS	IMM	IMS
CM	1	0.77	0.76	0.78	0.78	0.79
CS	-	1	0.77	0.77	0.77	0.78
BM	-	-	1	0.76	0.80	0.79
BS	-	-	-	1	0.80	0.79
IMM	-	-	-	-	1	0.80
IMS	-	-	-	-	-	1

et al. 2012). Results obtained in our study indicate that the application of only one controlled burn, regardless of its timing, is not enough to control *M. minutiflora* in this area of Central Brazil. When compared to the values recorded before the intervention, our field observations showed that a period of two years was sufficient for a fairly complete recovery of the biomass of *M. minutiflora*, due to both vegetative re-growth of the sprouted individuals and seedling emergence from the seed bank. The resilience after fire shown by *M. minutiflora* in our experiments corroborates observations by Filgueiras (1990) and Martins *et al*. (2004) that controlled burns are not an effective instrument for its control. However other studies Aronovich & Rocha (1985), Curado & Costa (1980), Bogdan (1977), and Williams & Baruch (2000) have shown that *M. minutiflora* can be eliminated by controlled burns.

Herbicides are a commonly used technique for control of invasive plants (Ortega & Pearson 2011) especially the herbicide glyphosate (Shaw *et al*. 2011). However, their use has contradictory opinions due to factors such as effectiveness on the target species, possibility of affecting non-target species and aspects related to contamination of the environment.

Under the studied conditions, the application of integrated management (in May or September) proved to be a promising technique for the control of *M. minutiflora*. In 2006 its biomass showed a decrease of 99 %, compared to its original value, and this represented only 0.60 % of the total ground layer biomass. In 2008, due to significant reinfestation, its biomass reached ca. 0.02 t/ha in the IMS plots and 0.045 t/ha in the IMS plots. In subsequent years, after manual removal, its biomass was lower than 0.01 t/ha for both treatments, being only 0.18 % of the *M. minutiflora* biomass values recorded before the interventions. Because of reinfestation by sprouting of seeds from the seed bank and possible dispersal from populations adjacent to the experimental area, further manual removal was necessary.

The use of the controlled burns facilitated the visualization of *M. minutiflora* for application of the herbicide but manual removal required more time due to regrowth of the native species. The time required to perform herbicide applications and manual removal of seedlings in the integrated management treatments of May and September varied from 12 to 15 minutes/100 m^2. Based on our data, we can estimate that a team of three workers would need approximately 8 hours near the end of the rainy season and prior to flowering to maintain one hectare free of *M. minutiflora*.

Biomass of the herbaceous and subshrub stratum

Introduced grasses generally show higher growth rates than those of the native species (Baruch *et al*. 1989; Aduan 1998). This is probably related to a higher photosynthetic capacity and more efficient allocation of its products in the formation of new leaves (Baruch *et al*. 1989; Aduan 1998). The biomass dynamics observed in areas invaded by *M. minutiflora* corroborates findings of Brooks & Pyke (2001), who observed that invasive plant species can alter the normal biomass values in the invaded areas. Similar results were also reported by Rossiter *et al*. (2003), Stanley *et al*. (2011), and Davies & Nafus (2013).

After re-colonization by the native vegetation, the biomass of ground layer in the integrated management plots in 2008 was about 5 t/ha, maintaining the same value until the last evaluation in 2013.The use of both burns and herbicides were essential in the success of the integrated management treatment. Monitoring of the glyphosate effectiveness showed that there was an 100 % mortality both of re-sprouted clumps and seedlings of *M. minutiflora*, established during the rainy season between December of 2003 and March of 2004, with no mortality of native species, since *M. minutiflora* was the only target.

In areas with an extremely high coverage (> 98 %) of *M. minutiflora*, and protected against fire for about two decades, its biomass values ranged from 12.1 to 21.4 t/ha. Our results are consistent with those obtained by Silva & Haridasan (2007) and Hoffman & Haridasan (2008), who reported a range of 8.7 to 15.7 t/ha of aerial biomass production for this grass. According to Miranda *et al*. (2004), the biomass of the native ground layer vegetation may vary from 6.9 to 10 t/ha, depending on the physiognomy of the Cerrado and the period of protection against fire. The biomass values of *M. minutiflora* recorded in this study were almost double of the values of the native species.

Species richness in the experimental area

Our study area, with an average *M. minutiflora* coverage of around 55 %, had an impressive floristic richness, which apparently was resistant to invasion by *M. minutifora*. The native vegetation had a high capacity for recovery, both through re-sprouting and germination from the soil seed bank after reduction of *M. minutiflora* in the integrated management plots. However, in the plots with higher coverage of *M. minutiflora*, some alteration in frequency and abundance of native species in the ground layer vegetation were observed, which may indicate the possibility of loss of species richness.

Even though *M. minutiflora* is considered the main invasive grass species of protected areas in Brazil, there have been no studies of *M. minutiflora* dealing with the threshold of its infestation ratio, above which its presence in the invaded areas affects the native species richness. During this study, the presence of *M. minutiflora* in subplots where its average biomass does not exceed 60 % of the total aboveground biomass, the native vegetation is apparently has the capacity to coexist.

A survey of exotic species in a protected area may show its susceptibility to invasion (MacDonald *et al*. 1986). In the PARNA Brasília 126 exotic species, among them 27 grasses, have been identified to the present time (Martins *et al*. 2007; Horowitz *et al*. 2013). Among the grasses, along with *M. minutiflora*, the species *Andropogon gayanus***,** *Hyparrhenia rufa*, and *Urochloa decumbens* are, all considered to be extremely aggressive and cited as invasive for the Brazilian Cerrado (Filgueiras 1990; Martins *et al.* 2011). These data support the importance of continual monitoring of protected areas to formulate possible management solutions for eradication or reduction of invasions.

From the start of our field experiments between one to five individuals of other invasive grasses *A. gayanus***,** *H. rufa*, and *Melinis repens* were found in the evaluated plots. The first record of *U. decumbens* dates to 2007, when it established just one focal point of colonization. In the same period, *A. gayanus* began its population expansion, occupying around 2 % of the CM plots and of the CS plots. Although these species were not the object of this study, manual removal of these species began in May of 2008, extending until 2010. Due to dispersal of various plantlet populations of *A. gayanus* and *H. rufa*, both present in the vicinity of the experimental area, their presence in the experimental areas was observed again in the period of 2011 to 2013 and the individuals were targeted for manual removal.

In conclusion, results of the BM and BS treatments show that a controlled burn by itself was not an effective technique to control *M. minutiflora*, at least under the present study condition. Due to its high capacity of re-infestation in the burned areas, two years were sufficient for its return to the biomass values recorded before the intervention.

The integrated management treatment (controlled burns/herbicide use/manual removal) either prior or after flowering of *M. minutiflora* proved to be a promising management technique for controlling *M. minutiflora* in our study area. Due to its great invading capacity, a long-term monitoring is recommended. Re-infestation foci, once identified, must be immediately eradicated in order to prevent the recolonization. Even though the integrated management technique proved successful for the control of *M. minutiflora* in the PARNA Brasília, it is essential that other management treatments are also evaluated.

Due to the lack of legal clarity in the Brazilian legislation for the use of herbicides in the protected areas, their managers are reluctant to allow research and/or use of management treatments with their use for the control of invasive species and their effects on native species. However, in light of the positive results presented in this study we hope that serious consideration will be given in the future for the use of glyphosate under controlled conditions for control of *M. minutiflora*.

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