https://doi.org/10.17648/heringeriana.v9i2.134

Jardim Botânico de Brasília

ISSN 1983-6996
Versão impressa

ISSN 2359-165X
Versão *on line*

Heringeriana

10(2): 79 - 90. 2015

ANNUAL MOWING PREVENTS THE RECRUITMENT OF MOLASSES-GRASS IN A BRAZILIAN SAVANNA

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ABSTRACT - *Melinis minutiflora* is an African grass that invades large areas of the Brazilian savannas. In this case study, annual mowing was used to prevent the recruitment of the invasive grass. In September 2007, after the dispersal of *Melinis minutiflora* seeds, an area of 50 x 50 m was mowed. In the following four years, to prevent recruitment of new individuals of *M. minutifolia*, the area was mowed before seed dispersal. Seed density of invasive and native species was determined from soil samples before each mowing. In 2007, the seed density of *M. minutifolia* varied from 8,025 to 22,350 seeds m^{-2} and, after 4 years, was reduced to 88 to 313 seeds m^{-2}. *Melinis minutifolia* recruitment was highest in the first growing season (8 seedlings m^{-2}) with no recruitment observed in the following years. The significant reduction in seed bank and seedling establishment indicates that mowing before seed dispersal is useful to control the recruitment of *M. minutiflora*.

Key words: cerrado; invasive species; seed density; seedling density

RESUMO (O corte anual reduz o recrutamento de capim-gordura em áreas de cerrado) – *Melinis minutiflora* é uma gramínea invasora em extensas áreas de Cerrado. Neste estudo de caso, foi utilizado corte anual a fim de verificar seu efeito no recrutamento de novos indivíduos de *Melinis minutiflora.* Em setembro de 2007, após a dispersão das sementes, o corte foi aplicado em uma área de 50 x 50 m e, nos quatro anos seguintes, o corte foi aplicado após a dispersão das sementes de *M. minutifolia.* A densidade de sementes no solo foi estimada antes de cada corte. Em 2007, a densidade de sementes de *Melinis minutifolia* variou de 8.025 a 22.350 sementes m^{-2}, sendo reduzida para 88 a 313 sementes m^{-2} em 2011. Na primeira estação de crescimento, o recrutamento foi de oito plântulas de *M. minutifolia* por metro quadrado, sem recrutamento nos anos seguintes. As reduções significativas no banco de sementes e no recrutamento sugerem que o corte antes da dispersão das sementes é útil para controlar o recrutamento de *M. minutiflora.*

Palavras-chave: Cerrado; densidade de sementes; densidade de plântulas; espécie invasora

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INTRODUCTION

Melinis minutiflora P. Beauv. (molasses-grass) is an African grass that was introduced as pasture in Brazil for its high nutritional value, and until recently was extensively used for restoring degraded areas. Nowadays it is considered a major invasive exotic species in Brazilian savannas, typical vegetation of central Brazil, especially in nature reserves since it excludes a large number of native species and reduces local diversity (Pivello *et al*., 1999; Martins *et al*., 2004; Freitas & Pivello, 2005). In natural areas, once the occupation by an invasive species is detected, long-term control and management programs must be implemented to eradicate the species and restore the area (Simberloff, 2009). Management may include the depletion of viable propagules in the soil bank by interrupting sexual reproduction (Zamora *et al*., 1989), which may require the understanding of the biology and population dynamics of the invasive species, since they exhibit phenotypic plasticity, the ability to reproduce sexually and/or vegetatively, rapid growth and tolerance to environmental heterogeneity (Baker, 1974; Simberloff, 2003; Sheley & Krueger-Mangold, 2003; DiTomaso *et al*., 2010).

In grassland areas, vegetation is commonly mowed in order to reduce the dominance of invasive species. The effectiveness of this methodology depends on the frequency and timing of mowing, as well as the height of cutting (Benefield *et al*., 1999; DiTomaso *et al*., 2010; Sato *et al*., 2013). However, after the mowing, natural recovery may occur through germination of propagules in the soil seed bank, vegetative propagation and/or seed dispersal from extant vegetation. The density of seeds in the soil bank can limit the recovery of native species since the dominance of invasive species can lead to the absence or low density of seeds of native species (Turnbull *et al*., 2000; Reinhardt-Adams & Galatowisch, 2008). Then, the study of the soil seed bank helps the understanding of the successional processes after disturbance or management (Csontos, 2007; Bueno & Baruch, 2011).

Removal, by burning or clipping, of the above-ground biomass of the native grasses of the Brazilian savannas usually stimulates flowering (Meguro, 1969; Coutinho, 1990; Munhoz & Felfili, 2005) and does not affect primary production (Nascimento-Neto *et al*., 1998). For *M. minutiflora*, defoliation does not stimulate flowering, and when this grass is clipped below 10 cm in height primary production is reduced and mortality of individuals is observed due to the apical position of the meristem (Caro-Costas & Vicente-Chandler, 1961; Klink, 1994). Therefore, mowing as a management technique to control *M. minutiflora* may benefit the native grasses. We evaluated mowing before *M. minutiflora* released its seeds as a management technique to prevent the addition of *M. minutiflora* seeds to the soil bank, reduce seedling recruitment and to stimulate the seed production of native grasses.

MATERIALS AND METHODS

Melinis minutiflora is an African grass that was introduced to Brazil in the 17th century. Until recently, it was used extensively as forage (Parsons, 1972; Williams & Baruch, 2000), but nowadays is replaced by more productive grasses such as *Urochloa* spp. and *Andropogon gayanus.* Able to spread rapidly, this grass species currently occupies large areas in several nature reserves in central and southeast Brazil (Pivello *et al*., 1999; Martins *et al.*, 2004; 2009). *Melinis minutiflora* is a stoloniferous perennial grass with ascending culms (0.40 to 1.60 m). Molasses-grass flowers annually (May to July) and disperses its seeds one month after flowering. Seed production is high (5,000 to 40,000 seeds m^{-2}) with a wide annual variability. Although the seeds show high viability (89% to 97%) and germinate after eight years of storage in laboratory conditions, the germination rate is low, from 6% to 27% (D'Antonio *et al*., 2001; Barger *et al*., 2003; Martins *et al*., 2009, Carmona & Martins, 2010).

The present case study was conducted in the Reserva Ecológica do IBGE (15°56'20''S and 47°53'16''W), 35 km south of Brasília in central Brazil. The climate is seasonal with well-defined dry (May to September) and rainy (October to April) seasons; about 90% of the mean annual rainfall of 1,436 mm occurs in the latter period (IBGE, 2004). The reserve harbors 186 native and 57 exotic grass species, including *M. minutiflora*. Molasses-grass is found in various parts of the reserve, especially in areas used until 1956 for extensive cattle and subsistence farming. Currently, dense stands of *M. minutiflora* occur near the edges of gallery forests and roadsides (Hoffmann *et al*., 2004; IBGE, 2004).

Along a roadside, an area (50 x 50 m) of campo sujo (a grassland with scattered shrubs) invaded by *M. minutiflora* was selected and mowed for the study. At the start of the study, in 2007, the area had been protected from fire for 5 years and the herbaceous layer coverage was 92.3%, and the herbaceous layer biomass was 7.1 ± 2.6 Mg ha^{-1}; molasses-grass accounted for 27.5% of the coverage and 9.8% of the biomass.

To ensure the highest density of seeds in the soil bank, the first mowing was done in September 2007, after *M. minutiflora* and most of the native grasses had dispersed their seeds (Almeida, 1995; Martins & Leite, 1997), and before the beginning of the rainy season, to avoid seed germination. Using a micro-tractor, the mowing height of the herbaceous layer varied from 7 to 10 cm due to irregularities in the terrain. The height of 10 cm was chosen since it is the usual mowing height used in cultivated pastures in the region (Klink, 1994), and the hay was left in the field after mowing. In subsequent years (2008, 2009, 2010 and 2011), mowing was applied during the molasses-grass flowering period but before its seed dispersal (June). Becker & Fawcett (1998) and Wilson & Clarke (2001) suggested that mowing is more effective if applied during flowering when the concentration of carbohydrates is higher in the aerial portion.

The density of germinable seeds in the soil bank was estimated annually, before mowing, from 10 soil samples (20 cm x 20 cm x 2 cm) that were randomly collected in the area. The depth of 2 cm was adopted since in the Brazilian savanna, the soil seed bank is superficial, with 90% of seeds located in the first centimeter of soil

(Andrade *et al*., 2002) and molasses-grass seeds that are below this depth rarely germinate (Martins *et al*., 2009). The soil samples were placed in plastic trays in a greenhouse. The samples were kept in ambient conditions and watered as necessary. Seedlings were counted, identified as molasses-grass or native grasses, and then removed; this was done twice a week for six months. When emerging seedlings were not observed for two weeks, the soil was stirred to stimulate a new flush of seedlings.

Seedling establishment in the area was monitored to evaluate the effectiveness of mowing in reducing the seed density of the invasive species. Annually, from January through May, seedlings were counted every two weeks, in five plots (50 cm x 50 cm -each) randomly located in the area. The plots were 10 m distant from the edge of the area, to avoid accounting from seedlings originated from seeds of the extant vegetation. All *M. minutiflora* and native-grass seedlings present in each plot were counted and not removed. Only the seedlings remaining in the plots at the end of the rainy season (May) were considered as established individuals.

Due to the difficulty of carrying out experiments in natural reserves in Brazil that involve environmental alterations this study was restricted to one site with no replications. However, the five-year period provided replication over time allowing an *a posteriori* comparison to validate our conclusions.

The data were tested for normality and homogeneity, and due to the high variability, the densities of the soil seed bank before and after the mowing were compared using the Kruskal-Wallis test (α=0.05) and Dunn's *a posteriori* test for comparison between years. Densities are represented by the median. The χ^2 test (α=0.05) was used to test the proportion of seeds of native and exotic grasses.

RESULTS

In September 2007, the total density of germinable seeds in the soil bank varied from 2,700 to 22,675 seeds m^{-2} and *Melinis minutiflora* showed the highest density (8,025 m^{-2}) of all the native species in the area. All samples collected contained seeds of the invasive species, which comprised almost 99% of the seeds in each sample. Seeds of native grasses were present in 70% of the samples, with a density of 38 seeds m^{-2}, significantly lower than for molasses-grass (p=0.0002).

For the samples collected before the mowing of 2008, there was a significant reduction (97%; p=0.0002) in the total soil seed density as well as in the density of molasses-grass. From 2008 to 2011, *M. minutiflora* seed density varied from 88 to 313 m^{-2}, with no difference between years (p=0.7203; Figure 1a). For native grasses, there was no difference between the density of germinable seeds before the mowing of 2007, and the densities observed in the subsequent years (Figure 1b).

In 2011, seeds of molasses-grass were present in 90% of the soil samples and accounted for 84% of the seeds in each sample, a significant decrease (p<0.0001) compared with the samples collected in 2007. From 2007 to 2011 there was no difference in the number of samples

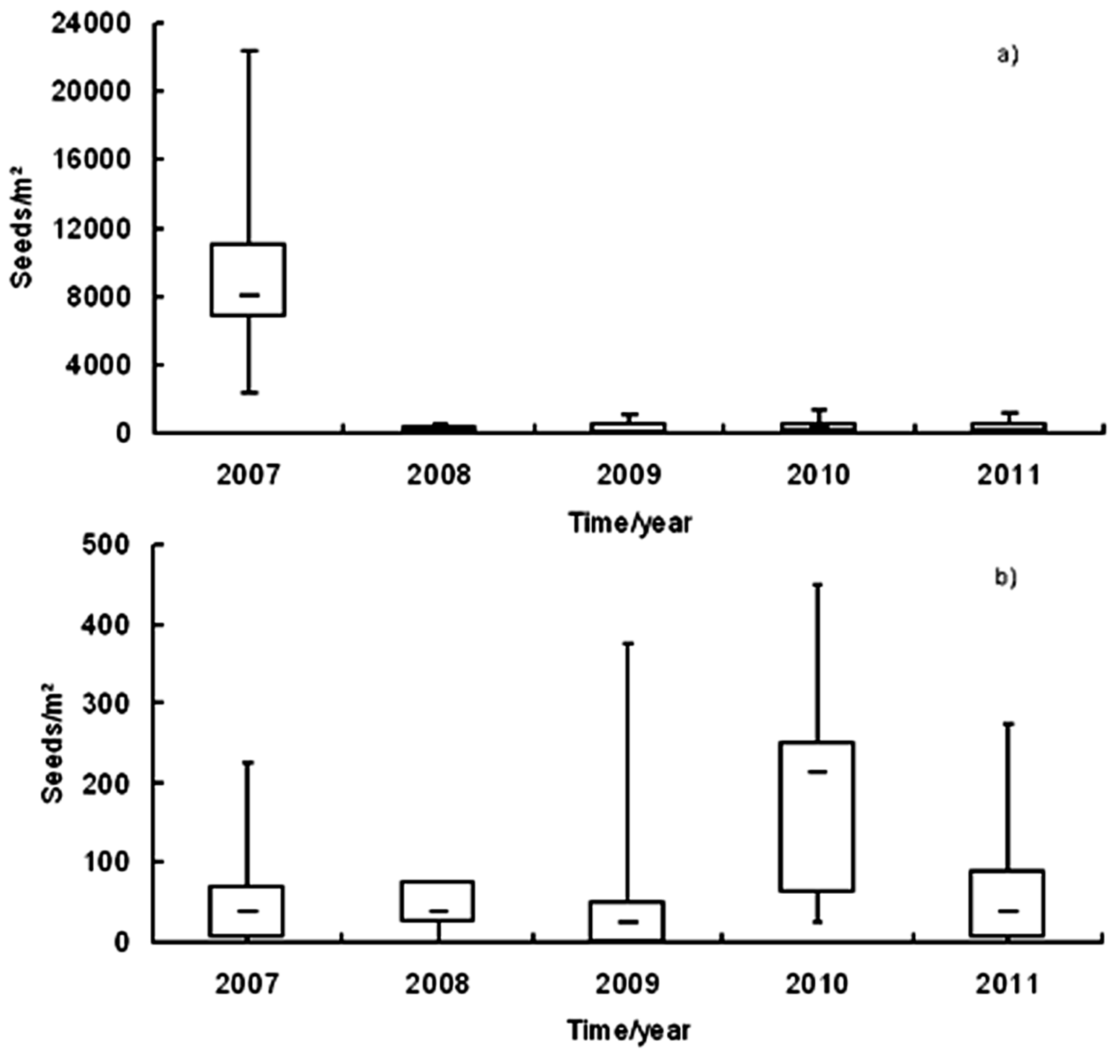

Figure 1. Soil seed bank of *Melinis minutiflora* (a) and native grasses (b) in a campo sujo area (open savanna) after the mowing of the vegetation at the Reserva Ecológica do IBGE, Brasília, Brazil. Mowing was carried in September 2007 after seed dispersal of the exotic species and from 2008 onwards always before seed dispersal of the exotic species (June).

containing seeds of native grasses; however, the contribution of native grasses to the total seed bank increased from less than 1% to 16% ($p<0.0001$).

In mid-rainy season of 2008, the maximum *M. minutiflora* seedling density was 264 m^{-2} (Figure 2a). In the wet season of 2009, the density was 36 seedlings m^{-2} and no seedlings were observed in 2010 and 2011. For native grasses, maximum seedling densities were 56, 104, 76 and 244 m^{-2} in 2008, 2009, 2010 and 2011, respectively. At the end of the rainy season (May) when seedlings in the area were considered established individuals, the mean density of native grasses was 16, 4, 0 and 16 m^{-2} in 2008, 2009, 2010 and 2011, with no difference between the years (Figure 2b). For the invasive species, new individuals were added to the area (8 m^{-2}) only in May 2008.

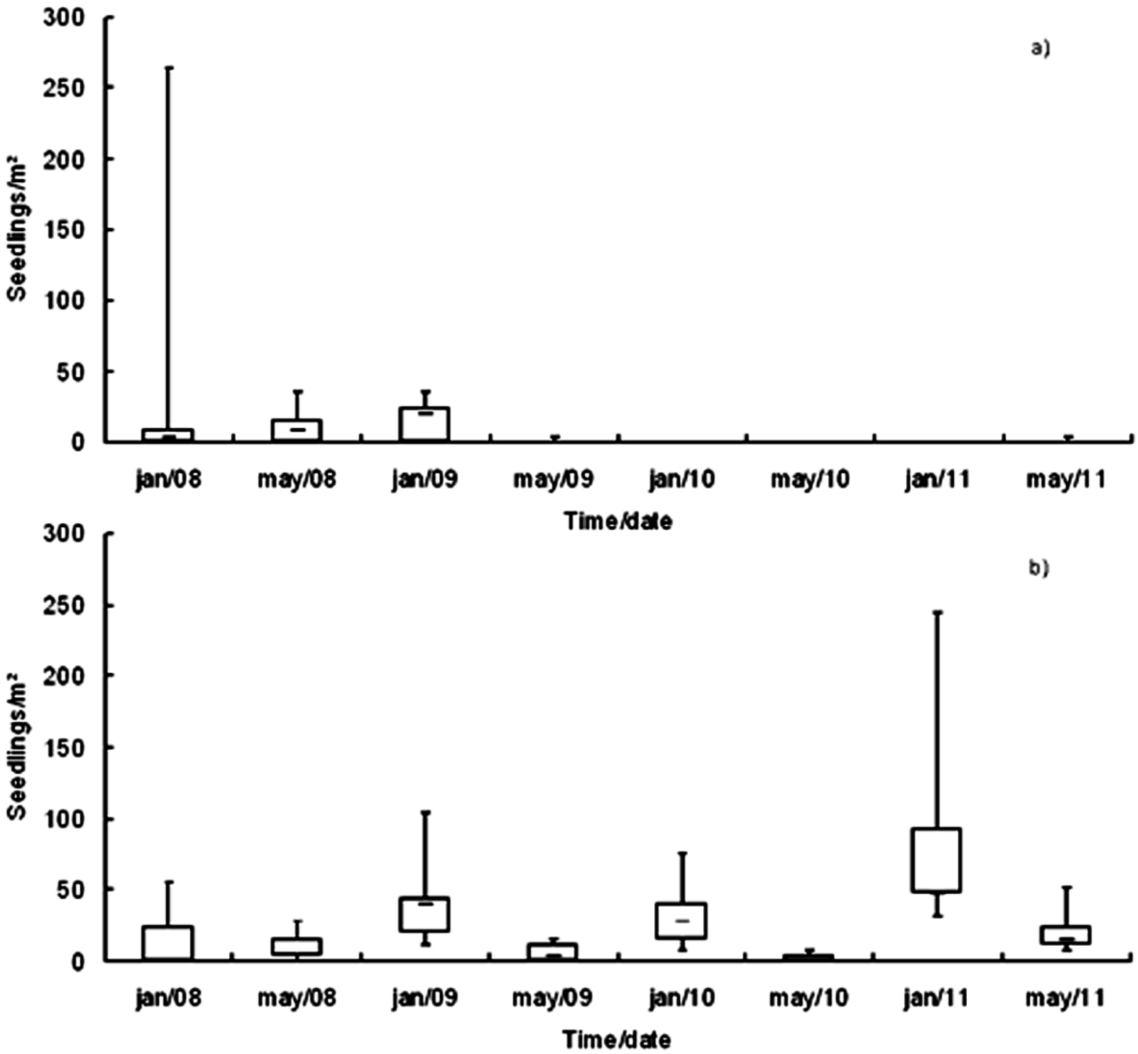

Figure 2. Seedling density of *Melinis minutiflora* (a) and native grasses (b) in a campo sujo area (open savanna) at the beginning (January) and end (May) of the rainy season at the Reserva Ecológica do IBGE, Brasília, Brazil. Mowing was carried in September 2007 after seed dispersal of the exotic species and from 2008 onwards always before seed dispersal of the exotic species (June).

DISCUSSION

The seed density of native species was in the range of values reported by Sassaki *et al*. (1999), Salazar *et al*. (2011) and Andrade & Miranda (2014) for different physiognomies of Brazilian savanna (37 to 144 seeds m^{-2}). Andrade & Miranda (2014) showed that the soil seed bank of native species is transient and is formed mainly by seeds of shrubs and herbaceous species that germinate in the beginning of the rainy season. The dominance of *M. minutiflora* seeds in the soil bank is due to its high seed production (Freitas & Pivello, 2005; Martins *et al*., 2009), combined with the high proportion of dormancy and long viability: 9 years in the laboratory (Carmona & Martins, 2010) and > 4 years in the field (this study) of the seeds, leading to permanent soil seed bank.

The reduction in the density of the soil seed bank observed from 2007 to 2008 was a consequence of the time of mowing and germination during the rainy season (October to April). In 2007, before the first mowing, *M.*

minutiflora had dispersed its seeds; therefore, the seed density of this species included both the seeds deposited on the soil surface and those stored in the first 2 cm of the soil. The effects of the 2007-2008 rains and the changes in the soil temperature due to removal of the vegetation cover may have stimulated the germination of molasses-grass seeds deposited on the soil surface. Martins *et al*. (2009) reported 83-85% germination rate of seeds of *M. minutiflora* deposited on the soil surface. Since there was no input of new seeds of *M. minutiflora* in the area, the similarity between the densities of germinable seeds from 2008 through 2011 suggests that the seed bank persisted as a consequence of the long-term viability of *M. minutiflora* seeds. During this period, the density (88 and 313 seeds m^{-2}) recorded in the molasses-grass soil bank was similar to the 337 seeds m^{-2} reported by Freitas & Pivello (2005) for a Brazilian savanna with different degrees of molasses-grass invasion in the Parque Estadual de Vassununga, southeastern Brazil. For native grasses, the similarity in the seed density recorded before the first mowing (2007) and from 2008 through 2011 may be a consequence of flowering triggered by shoot removal (Meguro, 1969; Coutinho, 1990; Munhoz & Felfili, 2005), which guarantees an input of seeds into the soil bank.

The low number of molasses-grass seedlings in the mowed area may be a consequence of the depth at which the seeds are buried and/or the low levels of radiation reaching the soil surface. Seeds of *M. minutiflora* below 2 cm depth rarely germinate (Martins *et al.*, 2009). However, as a result of irregularities in the terrain, during mowing the soil may be disturbed, exposing the seeds and favoring germination. The establishment of molasses-grass in areas of disturbed soil such as roadsides and mining areas has been widely reported (Hoffmann *et al*., 2004; Mortensen *et al*., 2009; Barbosa *et al*., 2010). With respect to changes in the radiation regime, D'Antonio *et al*. (2001) reported that seed germination is reduced by 50% when radiation reaches levels below 3% of full sunlight and that seedlings are intolerant to such low levels of radiation. Similar low levels of radiation may have occurred in the study area as a result of the rapid recovery of vegetation during the rainy season (Coutinho, 1990; Nascimento-Neto *et al*., 1998) and shading caused by the hay left in the field after mowing. Therefore, the reduction in the seed bank and the low number of seedlings of *M. minutiflora*, added to the mortality of individuals when the clipping height is lower than 10 cm (Caro-Costas & Vicente-Chandler, 1961; Klink, 1994) and the inability to recover pre-mowing architecture, with a significant reduction in height and in the number of live tillers (Klink, 1994), may enhance the effectiveness of the mowing method. Sato *et al.* (2013) working in the same area showed that annual mowing applied for five years altered the importance value of molasses-grass from the highest importance (35.9%) to the seventh more important (12.6%) with a reduction in molasses-grass cover associated with an increase in the native grasses cover.

The lack of change in the seed density of native species in the soil bank and in the density of established seedlings in the area suggests that this mowing regime did not affect the seed production of native grasses since for many native grasses in the Brazilian savanna, vegetation disturbance during the dry season, such as fire, results in earlier flowering period (Coutinho, 1990; Munhoz & Felfili, 2005) or rapid recovery of aboveground biomass with increased flower production (Meguro, 1969; Coutinho, 1990; Miranda & Klink, 1996; Parron & Hay, 1997), even in the absence of rain.

Despite the importance of seed availability for restoration success, applying a chosen treatment consistently at the same season and frequency can change the species' composition of the soil seed bank, and as a result, this practice may prevent species that disperse their seeds from mid- to late dry season from adding their seeds to the seed bank. For this reason, sowing seeds of native grasses with different phenological patterns in the area being restored may be an alternative to increase the richness and enhance the establishment of native species (Marrs & Lowday, 1992; Tilman, 1997; Turnbull *et al*., 2000; Aires *et al.* 2014) and increase competition with the invasive species (Tilman, 1997; Crawley *et al*., 1999).

CONCLUSION

The annual mowing applied to the campo sujo vegetation invaded by *M. minutiflora* reduced its seed density in the soil bank, without affecting the seed bank of native grasses. Although a certain amount of *M. minutiflora* seeds remained in the soil bank at the end of the fifth year of treatment, from the third year onward, no new individuals of *M. minutiflora* or decreases in the recruitment of new individuals of native grasses were observed, suggesting that the mowing scheme was effective in controlling the invasive species while benefiting the native species present in the area. The remaining seed bank and the long period of viability of the exotic species seeds suggest that mowing before the dispersal of the *M. minutiflora* seeds must be continued without interruption for at least nine years. Also, it is important to consider that the area mowed is large enough to prevent the dispersal of seeds of other invasive species that may be present in the adjacent vegetation. However, due to different phenological patterns of the native grass species, always mowing at the same season and frequency can change the soil seed bank composition of these species, which over the long term may reduce the diversity in the area. Nonetheless, the annual mowing may be applied along the roadsides in nature reserves to prevent the spread of *M. minutiflora.*

ACKNOWLEDGEMENTS

We gratefully acknowledge the financial support provided by PETROBRAS under Rede Temática "Conservação e Recuperação de Ecossistemas e Remediação de Áreas Impactadas" (resolução ANP n° 33 de 24.11.2005). We are also grateful to the staff of the Instituto Brasileiro de Geografia e Estatística (IBGE) for their authorization to carry out this study.

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