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Response of vegetation to sheep dung addition in a degraded Cerrado area

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ABSTRACT: Cerrado is the second largest biome in Brazil and is classified as a biodiversity hotspot. The establishment of hydroelectric power stations in Brazil originated degraded areas due to the removal of soil for construction of dams, in which native vegetation sometimes fails to reestablish due to the bad soil conditions. Sheep excrete most of the nutrients they ingest, such as phosphorus, calcium, potassium and nitrogen. This study aimed at investigating whether sheep dung contributes to the improvement of soil quality and stimulates the process of ecological succession in areas affected by the construction of the hydroelectric power station of Ilha Solteira. Four areas were selected, located at the Experimental Farm of Unesp Ilha Solteira/SP, and ten plots of 1 m^2 each were established. From October 2014 to December 2015, five replicates received 150 g fresh sheep dung every 15 days. In December 2015 soil sample from top 0.10 m was collected for the determination of soil attributes. Aboveground biomass was also collected, separated into four functional groups, oven-dried and weighed. Data were analysed to check the effect of dung addition upon soil and vegetation variables. Dung addition stimulated the development of several functional groups and changed soil nutrient concentrations in all four studied areas.

Key words: recuperation, restoration, nutrients

Resposta da vegetação à adição de esterco ovino em uma área degradada no Cerrado

RESUMO: O Cerrado é o segundo maior bioma do Brasil, sendo este um 'hotspot' de biodiversidade. A construção de usinas hidrelétricas dá origem a áreas degradadas, muitas vezes desprovidas de vegetação, denominadas "áreas de empréstimo". Ovinos excretam grande parte dos nutrientes que ingerem, entre eles fósforo, cálcio, potássio, nitrogênio. Este trabalho teve como objetivo investigar se a introdução de esterco de ovinos contribui para a melhoria na qualidade do solo e para o estímulo ao processo de sucessão primária em áreas de empréstimo da Fazenda Experimental da Unesp de Ilha Solteira. Foram selecionadas quatro áreas na Fazenda Experimental da Unesp de Ilha Solteira, em cada área foram estabelecidas dez parcelas de 1 m^2. De outubro de 2014 a dezembro de 2015, cinco parcelas receberam 150 g de fezes frescas de ovelhas a cada 15 dias. Em dezembro de 2015 coletou-se uma amostra dos primeiros 0,10 m de solo para a determinação de atributos do solo. Foi também coletada a biomassa aérea de cada parcela a 5 cm do solo, estas amostras foram triadas em quatro grupos funcionais, secas e pesadas. Dados foram analisados quanto ao efeito da adição de esterco sobre as variáveis medidas. Verifica-se que a introdução de fezes ovinas na área de empréstimo estudada estimulou o desenvolvimento de diversos grupos funcionais e modificou a concentração de nutrientes no solo em todas as áreas.

Palavras-chave: recuperação, restauração, nutrientes

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Introduction

The Cerrado is the second largest biome in Brazil, occupying 22% of the Brazilian territory, with phytophysiognomies that vary from open grasslands to forests (Ribeiro & Walter, 1988). It is considered as a biodiversity hotspot due to its enormous biodiversity, high degree of endemism and high susceptibility to human influence (Myers et al., 2000).

In some areas in the Cerrado, the upper soil layers were removed to be used in the construction of hydroelectric power stations, which created areas where the total vegetation was removed, as well as the seed bank (Alves & Souza, 2008). The removal of approximately 8.6 m of the original soil profile for the construction of the foundation of the dam of the hydropower plant from Ilha Solteira - Usina Hidrelétrica de Ilha Solteira - effectively eliminated the vegetation of a massive area at the Experimental Farm of Unesp Ilha Solteira, located in the municipality of Selvíria in the State of Mato Grosso do Sul (Alves et al., 2012). This procedure has created edaphic and microclimatic characteristics that jeopardize the process of natural succession in the area. Limitations of nutrients and/or water seem to hamper ecological succession in the studied area, since it is colonized by the bryophyte *Brachymenium exile* (Gomes Júnior et al., 2015), but plants forming higher vegetation are still very scarce.

Sheep excrete most of the nutrients they ingest. Most of the phosphorus (P) and calcium (Ca) are released in their dung (> 95%), potassium (K) is released via urine (70-90%), whereas nitrogen (N) is more evenly distributed between dung and urine (45-80%) (Whitehead, 2000). Therefore, sheep dung can be an important source of nutrients to the soil (Barrow, 1987; Williams & Haynes, 1990). In Scotland, it was shown that artificially introduced sheep dung caused an increase in phosphorus, nitrogen, organic carbon, calcium, magnesium and potassium in the soil (Shand & Coutts, 2006). In England, the introduction of sheep dung promoted increases in plant species richness, diversity and abundance in abandoned agricultural fields, which can be attributed to a high amount of insects attracted by the dung (Gibson et al., 1987). The effects of this important nutrient and moisture source, however, have never been studied to improve soil quality and help in the recovery of degraded lands in tropical areas.

The permanent degradation of natural areas is ethically incorrect and constitutes a major socioeconomic loss for the coming generations, therefore it is necessary to break this process and to recover such areas. This research aims at investigating whether sheep dung introduction improves soil quality and stimulates primary succession in areas that are impacted by the construction of the hydroelectric power plant of Ilha Solteira, SP, located in the Experimental Farm of Unesp Ilha Solteira, in Selvíria, MS.

Material and Methods

This study was performed at the Experimental Farm of Unesp Ilha Solteira, located in the municipality of Selvíria, in the State of Mato Grosso do Sul, Brazil. In that area, there is degraded land in various successional stages, including grassland, forest, *Brachiaria* fields and exposed soil (Figure 1). This region is located at the right margin of the Paraná river and has a humid tropical climate with a pronounced dry season from May to September and a rainy season that concentrates more than 70% of the rainfall from October to April. Soils are mostly composed of strongly acid Latosols (US classification: Oxisols). They are well-drained, deep, red, clay-rich, structurally strong but poor in mineral nutrients (EMBRAPA, 2013).

In October 2014, four areas were selected within the region shown in Figure 1, containing different phytophysiognomies varying in function of the degree and type of plant cover, as shown below:

Area 1: Heavily compacted soil (soil mechanical resistance to penetration = 3328 kPa) with no vegetation (Figure 2A);

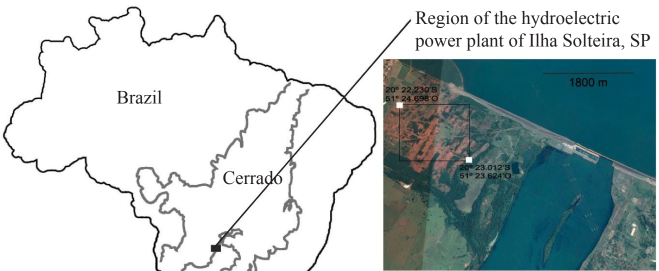
Area 2: Compacted soil (soil mechanical resistance to penetration = 2886 kPa) with sparse vegetation (Figure 2B);

Area 3: Forest border with plant cover formed by herbaceous and shrubby vegetation (soil mechanical resistance to penetration = 503 kPa) (Figure 2C);

Area 4: Grassland dominated by the exotic grass *Urochloa decumbens* (soil mechanical resistance to penetration = 580 kPa) (Figure 2D).

In each area ten 1 m^2 plots were established with minimum buffer of 1 m. From October 2014 and December 2015, five of the ten plots received 150 grams of fresh sheep dung every 15 days, which corresponds to an event of defecation of a medium size sheep (45 kg), whereas the other five plots were controls. Nutrient concentrations in the dung were determined according to the standard methods in the Plant Nutrition Lab from Unesp Ilha Solteira (Laboratório de Nutrição de Plantas da Unesp Ilha Solteira).

In December 2015 soil samples were collected in the 40 experimental plots. For each of these plots, three top-10 cm soil cores (5 cm diameter) were randomly collected and pooled to form a composite sample per plot. After drying at 70 °C samples were used to determine organic matter, pH, sum of bases, cation exchange capacity, base saturation and average concentration of available phosphorus, potassium, calcium, magnesium, sulfur, boron, copper, iron, manganese, zinc and aluminum, according to the standard methods used at the Soil



Area	Latitude	Longitude
1	20º23.012'S	51º23.624'W
2	20º22.550'S	51º24.347'W
3	20º22.541'S	51º24.302'W
4	20º22.448'S	51º24.404'W

Figure 1. Study area: Illustrative map of the study area with details of the Cerrado biome and the location of the degraded area at the Experimental Farm from Unesp Ilha Solteira in the municipality of Selvíria in the State of Mato Grosso do Sul. Latitude and longitude data shown in the table represent the central coordinates of each of the four studied areas

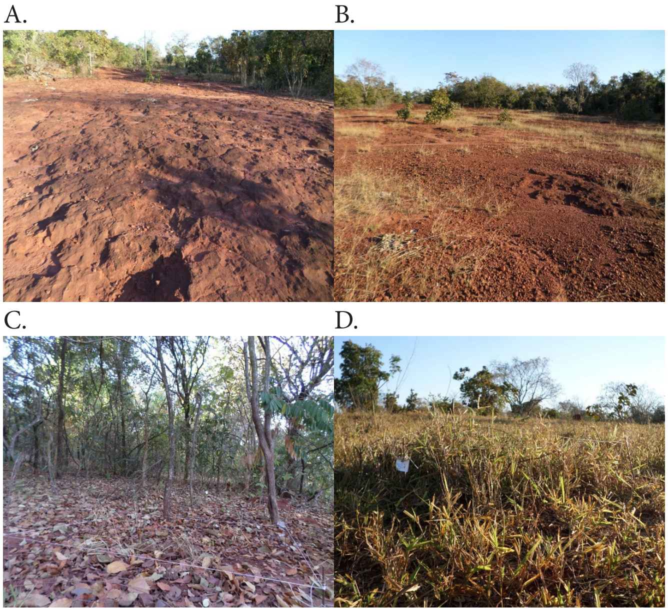


Figure 2. Areas selected for this study: Photographs of the four selected areas with the degraded field at the Experimental Farm of Unesp Ilha Solteira in Selvíria, MS: Area 1 (A); Area 2 (B); Area 3 (C); Area 4 (D)

Fertility Laboratory from Unesp Ilha Solteira (Laboratório de Fertilidade do Solo da Unesp Ilha Solteira).

Aboveground biomass of the 40 plots was harvested in December 2015. Nine months after this first harvest biomass was harvested for the second time. In both occasions, the biomass was sorted out in four selected functional groups (forbs, legume herbs, native grasses and exotic grasses). The reason for sorting the biomass out is due to the fact that these are the main groups found in Cerrado grasslands and that these functional groups respond differently to nutrient addition in the Cerrado (Bustamante et al., 2012; Lannes et al., 2016). This material was dried at 60 °C to constant weight, and weighed.

ANOVA and Tukey test were used to compare controls among areas and Student's t-test to check the effect of sheep dung addition on biomass production within areas, for total biomass and for each single functional group for both periods of biomass collection. Student's t-tests were also used to check the effect of sheep dung deposition on such soil comparing controls and treated plots. All statistical analyses were done using R version 3.1.1 (R Core Team, 2014).

Results and Discussion

Nutrient concentrations of the sheep dung used in the study are shown in Table 1. Concentrations of N, P, K, and other nutrients are higher than those of aquatic macrophytes used with the same purpose that yielded positive effects on plant cover in an adjacent area (Boni, 2017).

Aboveground biomasses were generally significantly higher in area 4 than in the other areas ($p < 0.001$), both in controls and treated plots. Areas 1, 2 and 3 had the same aboveground biomass, both in controls and in plots treated with sheep dung.

Sheep dung addition promoted changes in soil attributes and stimulated the development of several functional groups in the studied area (Tables 2, 3 and 4). Plant biomass responded significantly to dung addition in two of the four areas, and showed a statistical tendency in another area (Tables 2 and 3).

Area 4 was the most affected in terms of aerial biomass, with this increase promoted by a high increase in the biomass of exotic grasses (Tables 2 and 3). In this area, soil phosphorus was strongly affected by dung addition, as well as areas 1 and 3 (Table 4). Several native plant communities are limited by one or more than two nutrients, and phosphorus is one of the most limiting nutrient in such communities (Davidson & Howarth, 2007; Elser et al., 2007; Harpole et al., 2011; Townsend et al., 2011; Fay et al., 2015). Cerrado plants are thought to be primarily limited by phosphorus (Olde Venterink, 2011; Copeland et al., 2012). This seems to be the case when observing that plant biomass increased as well as phosphorus concentration in area

Table 1. Nutrient concentrations of the sheep dung used in the experiment

N	P	K	Ca	Mg	S	Cu	Fe	Mn	Zn
g kg^{-1}						mg kg^{-1}			
26.5	15.2	19.1	20.6	6.6	3.5	55	2931	254	406

Table 2. Mean aboveground biomass (g m^{-2}) of control plots (CT) and plots treated with sheep dung (SD) every 15 days, 12 months after fertilization with SD

Areas	Total		Exotic Grasses		Native Grasses		Legumes		Forbs	
	CT	SD	CT	SD	CT	SD	CT	SD	CT	SD
1	0	0.6^{t}	0	0	0	0.3	0	0	0	0.3^{t}
2	10.1	47.5*	0.1	2.7	10.0	38.5*	0.1	2.3	0	3.9*
3	3.4	3.4	1.6	0.3	0.3	0.5	0.9	0.2	0.7	2.2
4	52.9	221.6*	48.5	207.6*	0.1	0.9	4.2	8.9	0.2	4.1*

Data are shown for the four studied areas, divided into four functional groups: Exotic grasses, native grasses, legumes and forbs. N = 5. Significant differences ($p < 0.05$) between treated and control according to Student's t-test are shown with * while statistical tendencies ($0.05 < p < 0.10$) are indicated with t

Table 3. Mean aboveground biomass (g m^{-2}) of control plots (CT) and plots treated with sheep dung (SD) every 15 days, 21 months after fertilization with SD

Areas	Total		Exotic Grasses		Native Grasses		Legumes		Forbs	
	CT	SD	CT	SD	CT	SD	CT	SD	CT	SD
1	0	0.7	0	0.7	0	0	0	0	0	0
2	3.9	28.6^{t}	0.8	0	3.1	21.0^{t}	0	0.1	0	7.5
3	0.3	0.8	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.6
4	41.3	162.5*	40.6	155.0*	0.1	0.2	0.1	0.5	0.4	6.8*

Data are shown for the four studied areas, divided into four functional groups: exotic grasses, native grasses, legumes and forbs. N = 5. Significant differences ($p < 0.05$) between treated and control according to Student's t-test are shown with * while statistical tendencies ($0.05 < p < 0.10$) are indicated with t

Table 4. Values of pH, organic matter, cation exchange capacity, sum of bases, bases saturation, and concentrations of macro- and micronutrients in control plots (CT) and plots treated with sheep dung (SD) after a period of 21 months of sheep dung addition in four experimental areas at the Experimental Farm from Unesp Ilha Solteira (Selvíria, MS)

Soil attributes	Area 1		Area 2		Area 3		Area 4	
	CT	SD	CT	SD	CT	SD	CT	SD
Phosphorus	1.0	14.6 *	1.2	1.6	4.0	6.2 *	3.8	7.2 *
Org. matter	8.0	7.8	7.6	7.2	19.0	22.4 *	17.2	18.8
pH	4.2	4.6 *	4.4	4.3	4.2	4.3	4.5	4.6 *
Potassium	0.5	1.9 *	0.7	1.1	1.2	1.5 *	0.8	1.0
Calcium	2.2	4.0 *	4.6	3.0	6.0	8.2 *	7.4	8.0
Magnesium	1.4	2.8 *	2.6	1.8	6.0	8.2 *	6.6	7.6
Aluminum	4.6	2.2	3.6	5.4	6.6	5.0	3.0	2.0
Sum of bases	4.1	8.6 *	7.9	5.8	13.2	17.8 *	14.7	16.5
Sulfur	14.6	21.0	20.2	19.0	4.0	4.6 *	4.2	4.6
Cat. exch. cap.	22.9	25.6 *	27.9	30.8	42.5	52.0 *	40.9	39.1
Base sat.	17.8	33.4 *	27.8	20.2	31.6	34.2	36.0	42.2
Boron	0.03	0.07*	0.02	0.04*	0.13	0.13	0.07	0.07
Copper	0.30	0.38 *	0.34	0.32	2.00	2.12	1.56	1.52
Iron	3.0	4.6 *	5.0	4.2	41.6	48.8 *	26.6	24.8
Manganese	4.2	3.0	5.6	3.7	34.1	41.6 *	20.7	20.8
Zinc	0.06	0.22	0.06	0.06	0.40	0.76 *	0.44	2.34

Significant differences according to Student's t-test ($p < 0.05$) between control and treated plots are shown with*
The concentrations are as follows: Phosphorus, sulfur, boron, copper, iron, manganese, and zinc: mg dm^{-3}; Organic matter: g dm^{-3}; Potassium, calcium, magnesium, aluminum, sum of bases, cation exchange capacity, $mmol_c$ dm^{-3}; Base saturation, %

4 (Tables 2, 3, 4). It is important to note, however, that this area is dominated by the exotic grass *Urochloa decumbens*, which is known to be limited by phosphorus (Lannes et al., 2012, 2016), but does not represent the native Cerrado flora. The presence of exotic grasses with phosphorus increase in the soil allows to corroborate that these plants are limited by phosphorus, as it seems to be the case in area 4 and observed in a previous study in the Cerrados from the Brazilian central plateau (Lannes et al., 2016).

Different nutrients limit the several functional groups that form the Cerrado flora, with exotic invasive grasses being limited by phosphorus, whereas native grasses are limited by other nutrient than N or P (Lannes et al., 2016). In this study, differences in responses of the functional groups to the treatment can be explained by this diversity in nutrient limitation. For example, in contrast to the area 4, total aboveground biomass increased significantly ($p = 0.034$) as the biomass of native grasses increased in the first harvest ($p = 0.042$) in area 2, and also increased the same way in the second harvest, though not significantly. There is still no consensus on which nutrient limits the growth of Cerrado native grasses, but limitation or co-limitation by N or P is excluded (Lannes et al., 2016). In this study, one can speculate that boron is a possible candidate to main limiting nutrient for these Cerrado native grasses, since it was the only nutrient that had its concentration increased in area 2 due to dung addition.

A recent mesocosms study showed that boron addition could increase root biomass of *Digitaria insularis*, a common native grass in the study area (Silva et al., 2017). Another study, performed in the field next to study area, showed that boron tends to limit the biomass of native Cerrado grasses, though not significantly (Silva et al., 2017); this experiment has been running since 2016, being fertilized and harvested every year aiming at checking whether significant effects of boron on native grasses is detected after the third year, as observed in other fertilization experiments (DiTomasso & Aarssen, 1989; Güsewell et al., 2002; Harpole & Tilman, 2007; Lannes et al., 2016).

Area 1 was the most affected by sheep dung addition, since it was completely barren before the dung treatment and had three out of the four functional groups along the experiment. It is important to point out, however, that only exotic invasive plants (*Urochloa decumbens*) were present at the end of the experiment. As similarly observed in another study, it seems here that this exotic invasive grass benefits from the native ones to guarantee its establishment and success (Lannes et al., 2012). Dung addition changed several soil attributes, especially phosphorus concentrations, which increased nearly 15-fold in Area 1 in relation to the controls (Table 4).

Though not showing plant biomass response, area 3 had several soil attributes changed due to dung addition. Some nutrients (sulfur, manganese and zinc) only increased their concentrations in this area. It is possible to speculate that dung introduction could have increased soil microbial activity through the remediation of the differing nutritional limitations of soil bacteria and fungi, increasing decomposition rates from the allochthonous (dung) and from the autochthonous (litterfall) material, contributing to a more diversified increase of nutrients in this area.

Several factors can limit or co-limit the development of vegetation, and appropriate temperature is one of the most important conditions for life. Temperature can limit life distribution of a species through changes in survival, reproduction, growth, and in the interaction with other life forms. Previous studies have measured extremely high temperatures, with the maximum reaching 50 °C (Boni, 2017). Water availability is another critical factor for plants, and the local soil condition does not allow good water retention. These factors are not less important than soil nutrients, and should be considered as relevant when trying to recover a degraded area.

It is important to conduct this experiment for a longer time, to be able to see a clearer pattern in relation to vegetation succession and so that the results can be more expressive in terms of statistical significance. Authors indicate that such studies have to be conducted for a longer time, so the plant community has time to get adapted to the new soil conditions and the indirect effects of the fertilization can be evident, which generally occurs about three years after the first fertilization addition (DiTomasso & Aarssen, 1989; Güsewell et al., 2002; Harpole & Tilman, 2007; Lannes et al., 2016).

Conclusions

1. The introduction of sheep dung stimulate growth of vegetation, contributing to the process of ecological succession in the degraded areas in the Experimental Farm of Unesp Ilha Solteira.

2. There is an annual change in the composition of functional groups in the area, which is a natural pattern observed in a process of ecological succession.

3. This study corroborates data from Lannes et al. (2016), showing that plants from different geographical origins are limited by different nutrients even in degraded areas, and sheds a light upon which nutrient might limit the growth of Cerrado native grasses: boron.

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