

SABRINA DA SILVA PINHEIRO DE ALMEIDA

**SUBSTITUIÇÃO DE PASTAGEM NATIVA POR BRAQUIÁRIA:  
IMPACTOS NA ESTRUTURA E FUNÇÕES ECOLÓGICAS DA  
COMUNIDADE DE ESCARABEÍNEOS (COLEOPTERA)**

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Entomologia, para obtenção do título de *Doctor Scientiae*.

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“A gente sempre deve sair à rua como quem foge de casa,  
Como se estivessem abertos diante de nós todos os caminhos do mundo.\  
Não importa que os compromissos, as obrigações, estejam ali...  
Chegamos de muito longe, de alma aberta e o coração cantando!”

(Mário Quintana)

A todos que me abriram a alma e cantaram comigo.

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## RESUMO

ALMEIDA, Sabrina da Silva Pinheiro de, D.Sc., Universidade Federal de Viçosa, setembro de 2010. **Substituição de pastagem nativa por braquiária: impactos na estrutura e funções ecológicas da comunidade de escarabeíneos (Coleoptera).** Orientador: Carlos Frankl Sperber. Co-Orientadores: José Henrique Schoereder e Antônio Bento Mâncio.

A conversão de áreas savânicas em pastagens exóticas tem crescido em diferentes regiões com o objetivo de aumentar a capacidade de suporte dos pastos para o gado. Essas mudanças são particularmente notadas no Cerrado, o segundo maior bioma brasileiro, considerado uma das áreas prioritárias para conservação da biodiversidade. O Brasil possui um dos maiores rebanhos bovinos do mundo e cerca de metade das pastagens brasileiras, compostas por pastagens nativas e pastagens exóticas, se localizam na mesma região de distribuição do Cerrado. Apesar das consequências óbvias para a comunidade de plantas nativas, as consequências ecológicas da conversão, para a fauna nativa ainda são pouco abordadas. O Cerrado é um bioma moldado pelo fogo, e além da conversão de pastagens, o manejo com fogo em pastagens nativas (realizado a cada dois anos pelos produtores) têm trazido preocupações a respeito desse manejo sobre a fauna de invertebrados, ainda pouco estudada quando comparado com estudos com plantas. Dentre os invertebrados, os besouros escarabeíneos (Coleoptera: Scarabaeidae: Scarabaeinae) são considerados bioindicadores valiosos dos distúrbios antrópicos. Além disso, esses besouros têm um importante papel no desempenho de funções ecológicas como remoção de fezes das pastagens e bioturbação do solo. Porém, apesar da reconhecida atuação dos escarabeíneos como agentes benéficos para as pastagens, o uso indiscriminado de parasiticidas no gado pode afetar negativamente os besouros escarabeíneos. A presente tese visou investigar o impacto da substituição de pastagem nativa por braquiária na estrutura da comunidade de escarabeíneos e nas funções ecológicas desempenhadas por esses besouros nas pastagens do Cerrado. Investigamos o manejo realizado em propriedades de gado de leite, abordando o tempo de introdução da braquiária e o uso do fogo no manejo das pastagens nativas, além do manejo do gado bovino leiteiro utilizando o parasiticida ivermectina. A respeito de mudanças na comunidade de escarabeíneos devido à introdução, encontramos menor número de indivíduos, espécies e biomassa em pastos introduzidos quando comparados com pastos nativos. A composição de espécies em pastos nativos também é distinta dos pastos introduzidos, que são dominados por poucas espécies abundantes. Esses resultados nos mostram que a conversão de pastagens pode gerar uma reestruturação da

comunidade de escarabeíneos, especialmente em termos de diversidade local e composição de espécies. Podemos ressaltar que a manutenção de pastagens nativas no Cerrado pode ajudar a prevenir a perda da biodiversidade na paisagem agro-pastoril do Cerrado. Além disso, avaliando os determinantes da comunidade de escarabeíneos dentro de cada sistema de pastagens (nativo e introduzido), observamos que a riqueza de espécies para ambos sistemas foram determinados pelo *pool* regional de espécies. Fatores como proporção de areia no solo, penetrabilidade do solo, áreas de entorno das pastagens e tempo desde o último distúrbio (fogo ou introdução de pastagem exótica) foram determinantes da abundância para ambos sistemas. Porém, a introdução de pastagens exóticas mudou os efeitos dos determinantes da comunidade com o meio ambiente quando comparado com pastagens nativas. Nós mostramos que a conversão de pastagens vai além de simples mudanças na cobertura vegetal: os determinantes também são alterados, mudando a relação da comunidade de escarabeíneos com o habitat. Finalmente, observamos que o uso da ivermectina não afeta diretamente as funções ecológicas (fezes removida e solo escavado), porém, a ivermectina “interrompe” a correlação positiva da remoção das fezes com o número de indivíduos e a riqueza de espécies. Dessa forma, as fezes tratadas com ivermectina foram menos atrativas para os escarabeíneos, reduzindo a atividade dos besouros. Nós sugerimos que a ivermectina pode causar intoxicação nos besouros, atuando parcialmente como uma “armadilha ecológica”. Nós concluímos que a substituição das pastagens nativas em pastagens de braquiária acarreta em perda de diversidade biológica, mudanças em características do solo e a uma estagnação das funções ecológicas desempenhadas pelos escarabeíneos devido ao uso de ivermectina e, portanto, acarretando em prejuízo para a paisagem agropastoril do Cerrado.

## ABSTRACT

ALMEIDA, Sabrina da Silva Pinheiro de, D.Sc., Universidade Federal de Viçosa, September, 2010. **Replacement of native to exotic pasture: impacts on dung beetle (Coleoptera) community structure and ecological functions.** Adviser: Carlos Frankl Sperber. Co-advisers: José Henrique Schoereder and Antônio Bento Mâncio.

The replacement of native grasslands and bush savanna by exotic pastures has been implemented in many different regions to increase the livestock carrying capacity. These changes are particularly noticeable in the Brazilian savanna (Cerrado), the second largest biome in Brazil and one of the biodiversity conservation priority areas in the world. Brazil has one of the largest bovine livestock in the world and around half of all Brazilian pastures, composed by introduced and native pasture, are placed in the Cerrado region. Although these changes have obvious consequences for plant community, the ecological consequences for the native fauna are poorly known. Cerrado is a fire-shaped biome and besides the introduction of exotic grasses, there is a recent concern about the use of fire as a management tool in native Cerrado grasslands (each two years) on inhabiting invertebrate fauna. There is still a lack of information about the impact of these factors on insect's communities when we compare studies about impact on plant communities. Among insects, dung beetles (Coleoptera: Scarabaeidae: Scarabaeinae) can be used as cost-effective indicators of anthropogenic disturbance. Furthermore, dung beetles provided important ecological function such as faeces removal from pastures and soil bioturbation. However, even performing those useful ecological functions for farmers, the irresponsible use of parasiticides in bovine livestock can affect negatively the dung beetles. Here, we investigate the conversion of native pastures to exotic pastures on dung beetle community structure and upon the ecological functions performed by these beetles in Cerrado pastures. We evaluate the agropastoral management in dairy farms through the time since the conversion and the fire management on native pastures and also, the management of cattle with the ivermectin parasiticide. As results, we found differences in community structure and species composition between pasture systems, and introduced pastures were dominated by few abundant species. Our results showed that the conversion of native grasslands to introduced pastures can trigger a restructuring of dung beetle communities, particularly in terms of local diversity, species composition and overall dung beetle activity. These results highlight the importance of maintaining native pastures in the Cerrado agropastoral landscape, to help prevent widespread loss of biodiversity and ecosystem

functions. Evaluating the drivers of dung beetle community within each pasture system (native and introduced), we found that for both pasture systems, richness was driven by regional species pool. Sand proportion, soil penetrability, surrounding habitat and time since disturbance were determinants of dung beetle abundance in both pastures systems. However, the introduction of exotic pastures changed the effects of the environmental drivers on dung beetle communities, probably through soil management practices. We showed that habitat replacement goes beyond changes in vegetation cover: exotic grass introduction changes community-environment relationship for dung beetles. Finally, we observed that the ivermectin use does not affect in a direct way the ecological functions (faeces removed and excavated soil). However, ivermectin use broke down the positive correlation of faeces removal with dung beetle abundance and species richness. Therefore, ivermectin-treated cattle faeces was less attractive to dung beetles and reduced dung beetle activity. We suggest that ivermectin can cause beetle intoxication, working as a partial “ecological trap”. We concluded that the replacement of native to exotic pastures leads to biodiversity loss, changes in soil characteristics and the stagnation of ecological functions performed by dung beetles due to ivermectin use and, therefore, causing disadvantage to agropastoral landscape of Cerrado.

## INTRODUÇÃO GERAL

A perda da biodiversidade nas florestas tropicais devido ao desmatamento é um fato conhecido e bem documentado em todo o mundo (Gardner *et al.* 2009). Mesmo que muitas savanas tropicais estejam sendo alvo de uma rápida e drástica transformação devido a ações humanas, os padrões de perda da biodiversidade nesses sistemas ainda são pouco entendidos e estudados (Grace *et al.* 2006, Lehmann *et al.* 2009). Por exemplo, poucos trabalhos têm visado o estudo das consequências da conversão das savanas tropicais por pastagens exóticas (Pivello *et al.* 1999, Fairfax & Fensham 2000) quando comparamos trabalhos realizados com desmatamento das florestas (Parr *et al.* 2002, Bond & Parr 2010). Isso talvez ocorra devido às sutis mudanças que ocorrem na estrutura da vegetação dominada por gramíneas e arbustos das savanas, e que são dificilmente detectadas por ferramentas de detecção como o Sistema de Informação Geográfica (SIG), e por isso mesmo recebe menor atenção dos estudiosos e da sociedade (Houet *et al.* 2009).

Cerca de 1/5 da população humana e a maioria dos rebanhos vivem em ecossistemas savânicos (Lehmann *et al.* 2009). A conversão de áreas savânicas dominadas por gramíneas e arbustos em pastagens exóticas têm crescido em diferentes regiões com o objetivo de aumentar a capacidade de suporte dos pastos para o gado. Essas mudanças são particularmente notadas no Cerrado, o segundo maior bioma brasileiro, ocupando cerca de 20% do território brasileiro (Alho 2005). O Cerrado é considerado uma das áreas prioritárias para conservação da biodiversidade por ser um dos biomas mais ricos em espécies e também um dos mais ameaçados do mundo (Myers *et al.* 2000, Oliveira & Marquis 2002), possuindo apenas cerca de 2% de seu território em áreas protegidas (Klink & Machado 2005). O Brasil possui um dos maiores rebanhos bovinos do mundo e cerca de metade das pastagens brasileiras, compostas por pastagens nativas e pastagens exóticas, se localizam na mesma região de distribuição do Cerrado (Martha Junior & Vilela 2002, Silva *et al.* 2006). Estudos recentes sugerem que mais da metade do Cerrado remanescente é agora ocupado por atividades agro-pastoris (Ratter *et al.* 1997, Mittermeier *et al.* 2000, Bond & Parr 2010).

O Cerrado é formado por um complexo mosaico de fitofisionomias nativas que variam desde o campo limpo (predominância de gramíneas, usadas tradicionalmente como pastagem), passando pelo campo sujo e cerrado *sensu strictu* até florestas (Cerradão e florestas ciliares) semi-decíduas (Oliveira & Marquis 2002). A maioria da

sua vegetação foi degradada e a conversão das áreas para agricultura e pecuária ocorreu a partir dos anos 60, com a construção de Brasília e incentivos do governo para ocupação da região do Centro-Oeste brasileiro (Silva 2000). A predominância da vegetação de gramíneas e plantas arbustivas favoreceu a conversão, por ser mais fácil desmatar essas áreas do que áreas de floresta (Ratter *et al.* 1997). Infelizmente, até os dias de hoje, existe a idéia de que usar o Cerrado para a expansão agro-pastoril do país é um modo de proteger a Amazônia e evitar seu desmatamento (The Economist 2010).

O Cerrado tem sido convertido em monoculturas de soja para produção de *commodities* (Queiroz 2009) e cana-de-açúcar para produção de etanol que podem acarretar consequências desastrosas para a biodiversidade (Scharlemann & Laurance 2008). Pouco ainda é sabido a respeito da influência mais sutil da troca de “capim por capim” que ocorre nos campos limpos, geralmente substituídos por gramíneas exóticas, como a braquiária (Pivello *et al.* 1999). Apesar das consequências óbvias para a comunidade de plantas nativas, as consequências ecológicas da conversão para a fauna nativa ainda são pouco abordadas (Trolle *et al.* 2007, Carrijo *et al.* 2008).

O bioma Cerrado é considerado um bioma moldado pelo fogo, portanto, um elemento essencial a qual suas espécies desenvolveram adaptações para sobreviver (Pivello 2006). Entretanto, mudanças nos regimes de fogo, incluindo o período, a frequência e a intensidade, podem potencialmente destruir a vegetação nativa e prejudicar a fauna (Klink & Machado 2005). Atualmente, existe uma preocupação a respeito do manejo feito com fogo em pastagens nativas do Cerrado, utilizada a cada dois anos, com o objetivo de estimular a rebrota das gramíneas para alimentar o gado (Mistry 1998).

O impacto do manejo com o fogo feito com frequência e em períodos incorretos do ano é bastante óbvio e bem estudado para as plantas (p.ex. Ratter *et al.* 1997, Pivello *et al.* 1999, Mistry 1998, Pivello 2006, Silva *et al.* 2006). Entretanto, os impactos sobre a fauna, especialmente a de insetos ainda não são totalmente esclarecidos. A comunidade de insetos é uma ferramenta importante para a avaliação de impactos antrópicos em ecossistemas tropicais, principalmente devido à sua relativamente fácil amostragem e rápida resposta a mudanças ambientais (p.ex. DeSouza *et al.*, 2003; Carrijo *et al.* 2008; Vasconcelos *et al.* 2009, Louzada *et al.* 2010).

Besouros escarabeíneos (Coleoptera: Scarabaeidae: Scarabaeinae) são considerados bioindicadores valiosos dos distúrbios antrópicos (Halffter & Favila 1993). Eles são capazes de perceber mudanças no solo devido às suas necessidades alimentares e hábitos de nidificação (Hanski & Cambefort 1991); mudanças na estrutura da vegetação (p.ex. conversão de habitat e fogo); além da composição da paisagem (Navarrete & Halffter 2008; Almeida & Louzada 2009, Louzada *et al.* 2010). Além disso, os besouros escarabeíneos são importantes elementos no desempenho de funções ecológicas como, por exemplo, dispersão secundária de sementes e ciclagem de nutrientes (Nichols *et al.* 2008). Ainda mais importante do ponto de vista dos fazendeiros, os escarabeíneos são capazes de remover o esterco das pastagens, reduzindo a rejeição da forragem por parte do gado; além de suprimirem parasitas do rebanho, tais como helmintos gastro-intestinais e a mosca-do-chifre (*Haematobia irritans*). Essas funções ecológicas, que passam a ser serviços ecológicos quando quantificados em termos financeiros (Nichols *et al.* 2008), foram avaliados em US\$ 380 milhões de dólares ao ano nos EUA (Losey & Vaughan 2006).

Apesar da atuação dos escarabeíneos como supressores de parasitas, o uso de forma não consciente de parasiticidas no gado é um assunto amplamente debatido no mundo (Bianchin *et al.*, 1992; Suárez 2002; Kryger *et al.*, 2005; Iwasa *et al.*, 2007; Lumaret *et al.*, 2007). Os parasiticidas mais amplamente utilizados são aqueles de amplo espectro, que podem combater tanto endoparasitas (p.ex. helmintos), como os ectoparasitas (p.ex. carrapatos). Um dos grupos mais utilizados, em forma injetável no gado, é composto pelas lactonas macrocíclicas, que englobam a ivermectina, doramectina e a moxidectina (Lumaret & Errouissi 2002). Os mamíferos não são capazes de metabolizar completamente a maioria desses parasiticidas, que são excretados nas fezes e ajudam a combater as moscas parasitas que se reproduzem nessas fezes (Suárez 2009).

O problema do uso indiscriminado ocorre quando esses parasiticidas começam a afetar uma fauna não-alvo, como é o caso de vários invertebrados que vivem no solo e utilizam as fezes bovinas como recurso alimentar e/ou de nidificação (Lumaret & Errouissi 2002). As consequências do impacto sobre essa fauna não-alvo ainda não são completamente estudadas e podem ser potencialmente desastrosas, causando perda da biodiversidade (Lumaret and Errouissi, 2002; Römbke *et al.*, 2010), além da perda das funções ecológicas prestadas por essa fauna não-alvo, especialmente para os fazendeiros (Wall & Strong 1987).

A presente tese visou investigar o impacto da substituição de pastagem nativa por braquiária na estrutura da comunidade de escarabeíneos e nas funções ecológicas desempenhadas por esses besouros nas pastagens do Cerrado. Investigamos o manejo realizado em propriedades de gado de leite, abordando o tempo de introdução da braquiária e o uso do fogo no manejo das pastagens nativas, além do manejo do gado bovino leiteiro utilizando o parasiticida ivermectina.

A tese foi dividida em três capítulos, que foram escritos no formato de artigos científicos. O primeiro capítulo procurou avaliar a comunidade de escarabeíneos encontrada em pastagens naturais, formadas por campo limpo de Cerrado, e em pastagens introduzidas de braquiária. Para isso, testou-se a hipótese de que a introdução de pastagens exóticas afeta negativamente a riqueza, a abundância, a estrutura e a composição de besouros escarabeíneos. Este capítulo encontra-se aceito para publicação na revista *Biotropica*.

No segundo capítulo foram investigados os determinantes da comunidade de escarabeíneos dentro de pastagens nativas. Foi verificado se esses determinantes da comunidade se alteravam e mudavam as relações da comunidade com o meio em pastagens introduzidas de braquiária. Para isso, testaram-se as hipóteses de que os habitats do entorno das pastagens, características do solo e tempo de distúrbio determinam as comunidades de escarabeíneos. Este capítulo está no formato da revista *Basic and Applied Ecology*.

No terceiro capítulo, avaliou-se o efeito da ivermectina (parasiticida) utilizada no gado bovino sobre a comunidade de besouros escarabeíneos em pastagens naturais e introduzidas; além disso, foi verificado se a ivermectina afeta as funções ecológicas realizadas pelos besouros escarabeíneos. Para isso, testamos as hipóteses de que o uso da ivermectina e os sistemas de pasto introduzidos afetam negativamente a comunidade de escarabeíneos e conseqüentemente a remoção de fezes e a bioturbação do solo. Este capítulo encontra-se no formato da revista *Agriculture, Ecosystems & Environment*.

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## CAPÍTULO 1

### **Subtle Land-Use Change and Tropical Biodiversity: Dung Beetle Communities in Cerrado Grasslands and Exotic Pastures**

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## ABSTRACT

Although many tropical savannas are highly influenced by humans, the patterns of biodiversity erosion in these systems remain poorly understood. In particular, the biodiversity consequences of the replacement of native grasslands by exotic pastures have not been studied. Here we examine how the conversion of the native savanna grasslands by exotic pastures affects dung beetle communities. Our study was conducted in 14 native (native grassland- *campo limpo*) and 21 exotic (*Urochloa* spp. monoculture) pastures in Carrancas, Minas Gerais, Brazil. We collected 4996 dung beetles individuals of 66 species: 3139 individuals of 50 species in native pastures and 1857 individuals of 55 species in the exotic pastures. Exotic pastures had lower dung beetle richness, abundance and biomass than native pastures. Species composition between the two pasture types was significantly different and exotic pastures were dominated by few abundant species. Indicator species analysis (IndVal) detected 16 species indicators of native pastures and three of exotic pastures according to relative abundance and frequency in each pasture system. Our results show that the conversion of native pastures to exotic pastures leads to a predictable loss of local species richness, increase of dominance and changes in species composition. These results highlight the importance of maintaining native pastures in the Cerrado agro-pastoral landscape.

*Key words:* agro-pastoral landscape; Brazil; Brazilian savanna; habitat conversion; native pasture; Scarabaeinae.

## INTRODUCTION

THE LOSS OF TROPICAL BIODIVERSITY DUE TO DEFORASTATION IS WELL DOCUMENTED (E.G. Gardner *et al.* 2009). Although many tropical savannas have undergone similar human-induced transformations as the forests, the patterns of biodiversity erosion in these systems remain poorly understood (Lehmann *et al.* 2009). For example, very few studies have addressed the conservation implications of converting native savannas to exotic pastures (Pivello *et al.* 1999) when compared to deforestation (Bond & Parr 2010), perhaps because the structural changes in grass-dominated ecosystems are less obvious and more difficult to detect using remote sensing (Houet *et al.* 2009).

One-fifth of the human population and most of the world's livestock lives in Savanna ecosystems (Lehmann *et al.* 2009). The replacement of native grasslands and bush savanna by exotic pastures has been implemented in many different regions to increase the livestock carrying capacity (Pivello *et al.* 1999, Jepson 2005). These changes are particularly noticeable in the Brazilian savanna (hereafter referred to as Cerrado). This Neotropical ecosystem covers around 20 percent of Brazil (Alho 2005), and is considered one of the 25 hotspots to biodiversity conservation in the world (Myers *et al.* 2000) due to the high rate of conversion and the occurrence of thousands of endemic animals and plants (Bagno & Marinho-Filho 2001, Oliveira & Marquis 2002, Hoffmann *et al.* 2004). At the same time, Brazil has one of the largest bovine livestock in the world (FAO 2008), and around half of all Brazilian pastures are composed of exotic grasses with the majority located in the Cerrado (Martha Junior & Vilela 2002).

Cerrado is a complex mosaic of native vegetation, including grassland (*campo limpo*), savanna (*cerrado sensu strictu*) and forest (Cerradão) (Oliveira & Marquis 2002). Most of the Cerrado vegetation was degraded or converted to agriculture in the 1960's. This was stimulated by the Brazilian government and helped by the construction of several roads that facilitated access to areas with low population densities (Silva 2000). Recent estimates suggest that more than a half of the Cerrado is now occupied with agro-pastoral activities (Ratter *et al.* 1997, Bond & Parr 2010).

The conversion of the Cerrado vegetation to intensive monocultures such as sugarcane for biofuel production can be expected disastrous consequences for biodiversity (Scharlemann & Laurance 2008). However, we have a poor understanding of the more subtle changes that occur when Cerrado grasslands are planted with exotic grass species. Although these changes would have obvious consequences for the plant

community, the consequences for the native fauna are poorly known and the two of few existing ecological studies provide information about two very different taxa. While Carrijo *et al.* (2008) found that some termite species disappear from exotic pastures after replacement, Trolle *et al.* (2007) found that the maned wolf (*Chrysocyon brachyurus*) was able to survive in a mixed agricultural landscape including both exotic pastures and natural habitats.

The objective of our work was to examine the biodiversity consequences of replacing native Cerrado grasslands that are currently used as extensive pastures with pastures planted with exotic grass. We focused on dung beetles (Coleoptera: Scarabaeidae: Scarabaeinae) as a study group as they are considered cost-effective indicators of disturbance (Halffter & Favila 1993, Nichols *et al.* 2009), and have a high degree of habitat specificity in the Cerrado (Almeida & Louzada 2009). Furthermore, dung beetles are closely linked to mammals because both adult and larvae use dung as a food resource (Hanski & Cambefort 1991). As a result, they undertake important ecological functions such as secondary seed dispersal and nutrient cycling (Nichols *et al.* 2008). Most importantly from the perspective of cattle farmers, dung beetles bury livestock dung, reducing both forage fouling and the abundance of some common parasites that affect livestock (e.g. haematophagous flies). These ecological services have been estimated to be worth around \$380 million/yr in the USA alone (Losey & Vaughan 2006). The specific hypotheses we set out to test were: (1) exotic pasture system has fewer species and individuals than native system; (2) total dung beetle biomass is smaller in exotic pasture; (3) there are differences in the dung beetle community structure and species composition between pasture systems.

## **METHODS**

**STUDY SITE** - The study was carried out in Carrancas, in the south of the State of Minas Gerais, SE Brazil (21°28'24" S, 44°39'05"W), situated in the Cerrado biome (Oliveira-Filho *et al.* 2004). The sample sites were between 900m and 1,200m in altitude, and receive 1,480mm of rainfall/yr with a mean annual temperature of 15° C (Oliveira-Filho *et al.* 2004). The study region is one of the most important milk producing regions in Minas Gerais (Zoccal *et al.* 2006), and dairy farming is the main economic activity in many of the small cities, including Carrancas (IBGE 2006), part of the traditional "Finest Cheese Circuit" in Brazil (Leandro 2008).

Almost all farmlands in Carrancas contain some native Cerrado grasslands (*campo limpo*), and traditionally the farmers utilize these native grasslands to graze their cattle (S. Almeida, pers. obs). These native pastures (*campo limpo*) are composed of several native species of grass (Poaceae) and many other plant families, including dicotyledon plants (Rodrigues & Carvalho 2001, Munhoz & Felfili 2006). However, in the last 30 years, exotic grasses have been introduced to increase the carrying capacity of cattle. These exotic grasses include the African species *Urochloa* (*Urochloa* P. Beauv. spp. (= *Brachiaria* (Trin.) Griseb. spp.) which is highly tolerant of acidic soils characteristic of Cerrado (Martha Júnior & Vilela 2002). The replacement process is associated with several technological changes in pasture management as ploughing and the use of fertilizers and lime, for example, which is not used in native pastures (Martha Júnior & Vilela 2002).

We sampled dung beetles in 35 independent pastures sites which were a minimum of 300 m apart. There were 14 pastures covered by native Cerrado grassland and 21 covered by *Urochloa* spp. grasses. The pastures were distributed across seven medium to large dairy farms in Carrancas, and all were used for grazing cattle (Fig. 1). The farm varied in size from 43 to 457 ha, which reflected the typical range of farm sizes registered in Carrancas (IBGE 2006).

**DUNG BEETLE SAMPLING** - All sampling was undertaken during the middle of the rainy season, in January 2008, in order to minimise the potential effect of seasonality in our comparisons across farming systems. The rainy season is recognised as the best period of the year to sample dung beetles in the seasonal tropics (Martínez & Vásquez 1995, Milhomem *et al.* 2003).

Our sampling unit was a baited pitfall trap composed of a plastic container (diameter 19 cm, height 11 cm) filled with 150 ml of a saline solution and detergent. The trap has a base made of wire in the form of a hoop to accommodate a small plastic container (diameter 4 cm, height 4 cm) where the bait was placed. The wire was fixed in the soil in a way that the bait container was suspended in the centre of the trap. We also used a small plastic cover (20 cm diameter) sustained by three sticks to protect the trap from rain.

We placed six traps in each pasture site, and these were distributed in a rectangular design with 100 m between traps (Fig. 1). Traps were baited and left in the field for a 48h period in each pasture. We placed a total of 210 traps in the study (six traps times 35 pastures). Traps were baited with 20 g of human faeces in order to attract

a wide range of species (Larsen & Forsyth 2005). Previous studies show that cow dung only attracts a limited suite of species, underestimating dung beetle biodiversity (Dormont *et al.* 2004, Louzada & Silva 2009). Furthermore, our study had the objective to assess the total dung beetle diversity in the landscape: those dung beetles that use native mammal faeces present in Cerrado and also the generalist dung beetles that are able to use both native mammal faeces and cow due to its predominance in native areas used as pastures, as *campo limpo*.

Dung beetles were identified to species by Dr. Fernando Z. Vaz-de-Mello or using the reference collection of Invertebrate Ecology and Conservation Laboratory (IEC) at Universidade Federal de Lavras, Brazil. Voucher specimens were stored at the IEC and Universidade Federal do Mato Grosso (UFMT) collection. Whenever sample sizes permitted, we weighed 30 individuals of each species (including both males and females in almost same proportion), drying all specimens in a constant-temperature oven at 40°C for one week prior to weighing on a precision scale (0.0001g). The mean species weight was multiplied by the species abundance to obtain an estimate of biomass (Peck & Howden 1984) per trap in each pasture systems. We also classified dung beetles in the following functional guilds relating to their nesting behaviour: (i) rollers; (ii) tunnelers; and (iii) dwellers (Hanski & Cambefort 1991).

**DATA ANALYSES-** We used individual-based rarefaction analysis to compare patterns of species richness and sample effort in native and exotic pastures (Gotelli & Colwell 2001). Comparisons among pasture systems were made by visual assessment of overlapping 95% CI of the rarefaction curves implemented in EstimateS7.5 (Colwell 2005).

We used a generalized linear model (GLM) to examine differences in richness abundance and total biomass between pastures sites in both pasture systems. We used Poisson error structure to richness and abundance, normal error structure to biomass and quasi-Poisson error structure when overdispersion was detected (Crawley 2007, Zuur *et al.* 2009). Minimal models were adjusted by excluding non significant variables and verifying effects on deviance (Crawley 2007). All values were converted to mean per pasture to reduce the overall variability and spatial pseudoreplication. In addition, we used non-parametric Kruskal–Wallis tests to examine the abundance variation of each dung beetle species in both pastures system due to non-normal distribution of the data. We compared the average body weight of species in the different pasture systems using non-parametric Kruskal–Wallis tests. We used a non-parametric Pearson’s correlation to

relate abundance with total biomass. All GLM and Kruskal–Wallis analyses were undertaken within the R environment (R Development Core Team 2008).

We plotted species rank-abundance distributions to visually compare patterns of species dominance in the two pasture systems. Species were ranked followed their mean relative abundance in the native pastures. We used non-metric multidimensional scaling (NMDS) to explore differences in community structure and composition in the 35 pastures. The NMDS was based on a similarity matrix constructed using the Bray-Curtis index on log-transformed abundance and presence/absence matrix. We used the same NMDS method to test differences of dung beetles guild composition between the two pasture types. The stress value is used to assess the robustness of the NMDS solution, with stress values above 0.2 indicating plots that could be unreliable (Clarke 1993). Analysis of similarity (ANOSIM; Clarke 1993) was used to test for significant differences in multivariate community structure. ANOSIM is a non-parametric permutations test for similarity matrices that is analogous to an ANOVA. These analyses were conducted in Primer v. 5 (Clarke & Warwick 2001).

We used the Indicator Value (IndVal) analysis (Dufrene & Legendre 1997) to identify the species that were significant and reliable indicators of each pasture system. The method combines data on relative abundance and frequency to assess the degree to which a given taxon is frequently associated with a particular habitat. Significant IndVal scores suggest that a given taxon is a faithful indicator of a certain habitat when contrasted with a distribution of indexes generated by Monte Carlo randomization procedure (5000 randomizations). IndVal analysis was implemented in PC-ORD5 (McCune & Mefford 2006).

## RESULTS

**RICHNESS AND ABUNDANCE ANALYSIS-** We collected 4996 individuals of 66 dung beetles species during the study, distributed across six tribes and 23 genera (Table S1): Ateuchini (22 species - 8 genera), Canthonini (24 species - 6 genera), Coprini (10 species - 3 genera), Eurysternini (1 species - 1 genera), Onthophagini (2 species - 1 genera), Phanaeini (7 species - 4 genera). In the 14 native grassland pastures we collected 3139 individuals of 50 dung beetles species. In the 21 exotic pastures we collected 1857 individuals of 55 dung beetles species. Species accumulation curves indicated no significant difference in overall species richness between native and exotic system (Fig. 2). However, mean species richness ( $\chi^2=14.20$ ,  $P<0.001$ ) and number of

individuals ( $\chi^2=9.76$ ,  $P<0.001$ ; Fig. 3) per pasture was higher in native than exotic pastures.

**TOTAL BIOMASS AND BODY WEIGHT-** Total biomass of dung beetles was higher in native pastures (mean  $\pm$  SE = 9.70 g  $\pm$  1.66) than in exotic (mean  $\pm$  SE = 4.42 g  $\pm$  0.79) pastures ( $F_{1,33}=10.69$ ,  $P<0.05$ ). Additionally, there was a marginally significant correlation between biomass and abundance ( $r=0.31$ ,  $N=35$ ,  $P=0.06$ ) but no correlation between biomass and richness ( $r=0.10$ ,  $N=35$ ,  $P=0.56$ ). There was no significant difference between the average body weight of the dung beetle species captured in native (mean  $\pm$  SE = 0.04 g  $\pm$  0.006) and exotic (mean  $\pm$  SE = 0.06 g  $\pm$  0.01) pastures (Kruskal–Wallis;  $\chi^2=0.09$ ,  $P=0.76$ ,  $df=1$ ).

**SPECIES COMPOSITION-** Almost all species were more abundant in native pastures (Table S1) than in exotic pastures. Exotic pastures are dominated by few abundant species (Fig. 4). Four of the five most abundant species (*Trichillum adjunctum*, *Canthidium barbaticum*, *Canthidium decoratum* and *Canthon virens*) were most abundant in native pastures (Table S1) but only three of these differences were significant: *C. barbaticum* ( $\chi^2=35.02$ ,  $P<0.001$ ), *C. decoratum* ( $\chi^2=52.99$ ,  $P<0.001$ ) and *Canthon virens* ( $\chi^2=27.79$ ,  $P<0.001$ ). Overall, the abundance of almost 40 percent of dung beetles species declined in response to exotic grasses, while just six percent increase with the replacement (Table S1). Of the 50 species captured in native pastures, 11 were only caught within that system. Of the 55 species recorded in exotic pastures, 16 were only collected in this pasture type and just one or two individuals of each species (Table S1).

Dung beetle community composition and structure were different between native and exotic pastures, with each pasture system forming a distinct cluster on the NMDS plot (Fig. 5; ANOSIM,  $R=0.22$ ,  $P<0.001$  for composition, and  $R=0.10$ ,  $P=0.05$  for structure). IndVal analysis highlighted 19 species as indicator species (at  $P<0.05$ ), around 36 percent of recorded species. Of these, 16 were considered indicators of native grassland and just three were indicators of the exotic pasture (Table 1). According to functional guild, we found 1588 individuals of 24 species of rollers group, five individuals of one species (*Eurysternus paralellus*) of dweller group and 3403 individuals of 41 species of tunnellers group (Table S1). There was no difference in the relative composition of the functional guilds between native and exotic pastures on NMDS plot (stress value=0.01).

## DISCUSSION

Land-use change has had an enormous impact on the Brazilian Cerrado over the past 30–50 yr (Silva 2000, Houet *et al.* 2009). Many of these changes are ongoing, but can often go unnoticed if they occur at fine scales or are not detectable by remote sensing (Peterson 2008, Houet *et al.* 2009). By investigating the consequences of the replacement of native pastures by exotic pastures on dung beetles communities in the Brazilian grasslands (Cerrado), we reveal the potential loss of biodiversity resulting from cryptic land-use change. This includes a marked decline in overall beetle abundance and species richness per pasture in the exotic system. We discuss these results, highlighting the importance of spatial scale and the conservation implications of the change of dung beetle community structure in exotic pastures.

SPECIES RICHNESS AT DIFFERENT SPATIAL SCALES - Human actions in managed landscapes can increase the regional diversity but have negative impacts on species richness at a local level (Estrada & Coates-Estrada 2002, Nichols *et al.* 2007). Although species richness per pasture (local scale) was much lower in the exotic pastures, this introduced system maintained a high overall species richness at the landscape level (regional scale). This result likely relates to the presence of only one or two individuals of some species in our samples, that could be transient species (Fagan *et al.* 1999) moving between the surrounding native vegetation which remains the predominant land-cover in the region (IBGE 2006, Scolforo *et al.* 2008). These movements are well documented for several beetles groups, including dung beetles (e.g., Grez & Prado 2000, French *et al.* 2001, Nichols *et al.* 2007) and this possibility was supported by the higher proportion of species with only one or two individuals collected in the exotic pastures. It seems likely that these exotic pastures were often used as stepping stone habitats by dung beetles dispersing in their search for food, preferential habitats, or as part of their reproductive strategy (Fagan *et al.* 1999, Estrada & Coates-Estrada 2002).

DUNG BEETLE COMMUNITIES IN CERRADO'S PASTURES- We recorded surprisingly few species typical of exotic pastures elsewhere in Brazil. For example, we found an overlap of only 16 species (29% of our samples) with exotic pastures in the Cerrado regions studied by Louzada and Silva (2009), and an overlap of just 22 species (33% of our samples) with the study of Almeida and Louzada (2009) in native habitats of Cerrado, including native grasslands not used as pastures, in the same region. As in this study,

both cited studies were conducted in January, and seasonality is unlikely to explain these results. Instead, the low degree of overlap could reflect a high beta diversity in open-systems in Cerrado (Almeida & Louzada 2009) or, most probably, the use human faeces instead of cow dung in this study, as the latter has been commonly used in studies to evaluate dung beetle species composition in exotic pastures elsewhere (Koller *et al.* 1999, Aidar *et al.* 2000, Marchiori 2000, Marchiori *et al.* 2003, Louzada & Silva 2009). Although we sampled pastures with a high availability of cow dung, we chose to use human faeces as bait to attract a wider range of beetle species dependent on the dung of native carnivores, herbivores and omnivores (Filgueiras *et al.* 2009).

It was surprising that no exotic dung beetles were found in this study. For example, *Digitonthophagus gazella* is an African dung beetle species exotic in Brazil, introduced during the 1980's to help control gastrointestinal helminths and the horn fly *Haematobia irritans* (Miranda *et al.* 2000) and has already been observed in several exotic pastures in Brazil (Koller *et al.* 1999, Aidar *et al.* 2000, Marchiori 2000, Marchiori *et al.* 2003), including the Amazon (Matavelli & Louzada 2008), but did not in South of Minas Gerais (Louzada & Silva 2009, Almeida & Louzada 2009). The reasons for its absence are not clear, but could relate to colonization time-lags.

THE EFFECT OF GRASSLAND CONVERSION ON THE DUNG BEETLE COMMUNITY - Deforestation and land-use change in forests landscapes often brings about stark changes in species composition and community structure (e.g. Barlow *et al.* 2007). The more subtle grass-to-grass land-use change in savannas has received much less attention (Bond & Parr 2010); it could also have important consequences for the diverse and endemic biodiversity found in Cerrado grasslands because the exotic grasses are able to invade and modify environmental conditions (Pivello *et al.* 1999).

Changes in dung beetle abundance can lead to a decrease of ecological functions important for pasture functioning, such as limiting the availability some inorganic elements (N, P, K) in the soil and reducing the primary productivity (Yamada *et al.* 2007, Borghesio 1999). Our results consistently indicate that almost all species of dung beetle had much lower abundance in the exotic pastures and 11 species were not collected in exotic pastures. These results were supported by the stark 40 percent decline in dung beetle abundance in exotic pastures. There are three complementary mechanisms which could explain the lower abundance of dung beetles in exotic pastures. First, savanna replacement would affect the availability and heterogeneity of food resources for dung beetles, as the disappearance of several plant species, including

Leguminosae families (Ratter *et al.* 1997), could affect the native mammal community activity on the area (Vieira & Baumgarten 1995, Vieira 1999). Also, a higher density of cattle means there is more herbivore dung, which could result in competitive advantages for a few species that can efficiently use this novel food resource (Louzada & Silva 2009). Second, the ploughing used when planting the exotic grass is likely to negatively affect dung beetles since most feeding galleries and nests are within the first 30 cm of the soil profile (Bang *et al.* 2005). Finally, the higher bovine densities at exotic pastures should result in soil compaction due to livestock trampling (Araújo *et al.* 2007) which may benefit the few species that are able to cope with the hardest soils (Halffter *et al.* 1992). Further studies are needed to examine the relative importance of these complimentary hypotheses.

DUNG BEETLE BIOMASS AND BODY WEIGHT- Dung beetles with a large body size and body weight are often the most likely to go extinct following land-use change (Larsen *et al.* 2005, Gardner *et al.* 2008). Our study showed that biomass and species body weight were not different between pasture systems, despite the fact that biomass was higher in native pastures, suggesting the link between body size and extinction risk may not be universal (see also Larsen *et al.* 2005). If biomass is related with land-use intensity then dung beetle biomass is likely to vary depending on how land-use change alters factors important for dung beetles, including resource availability, changes in soils, vegetation structure and temperature (see also Verdú *et al.* 2006, Nichols *et al.* 2007).

CONSERVATION IMPLICATIONS- Our results highlight a poorly understood threat to dung beetle biodiversity in Brazilian Cerrado. This is especially relevant, as Brazilian government departments often provide incentives for converting native grasslands into exotic pastures, with the aim to increase the pastures carrying capacity (Martha Júnior & Vilela 2002) and reducing of the use of fire management by farmers (Heringer & Jacques 2002).

The incentives regarding fire management are particularly interesting. Although farmers in the region are permitted to use fire to manage their native pastures under certain conditions (Minas Gerais 2004), this permission can take a long time to be obtained from the State institution responsible for licensing and fire monitoring (S. Almeida, pers.obs.). The difficulty of obtaining permission in the right period of the year for burning provide a perverse incentive for farmers to convert native pastures to the exotic pastures which do not require fire management (S.Almeida, pers.obs.).

Changing the governmental policies of subsidies for native pastures replacement, and making the legal fire management less bureaucratic, would help prevent biodiversity loss in Cerrado grasslands.

**CONCLUSIONS-** Although exotic pastures were not devoid of a native dung beetle fauna, we show that they contain a marked lower abundance and altered species composition when compared to the native pastures in the same region. Our results therefore highlight the importance of maintaining native pastures in the Cerrado agro-pastoral landscape. They reveals how the ongoing conversion of native pastures into exotic pastures is causing changes in dung beetle communities which could have possible cascading effects on the important ecological services provided by these insects. Although subtle changes in landscape structure and function may be more difficult to detect and may not attract as much attention as more drastic structural changes (such as deforestation), our findings emphasize their potential importance for the conservation biodiversity associated with tropical savannas.

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Table 1. Indicator species of two pasture types calculated with IndVal (related with species frequency and relative abundance), significance level (\* $P < 0.05$ , \*\* $P < 0.005$ , \*\*\*  $P < 0.001$ ), mean abundance and standard error (SE) trap per system. (N) indicates species indicator in native pastures and (I) in exotic pastures.

Species	System indicator	<i>P</i>	Native			Introduced		
			Abundance	SE	Ind Val	Abundance	SE	Ind Val
<i>Agamopus unguicularis</i>	N	*	0.85	0.20	64	0.27	0.07	8
<i>Agamopus viridis</i>	N	*	0.36	0.09	31	0.05	0.05	1
<i>Canthidium barbaticum</i>	N	**	3.00	0.52	70	0.98	0.24	13
<i>Canthidium decoratum</i>	N	**	7.50	1.06	80	1.81	0.28	15
<i>Canthidium marseuli</i>	N	**	0.25	0.06	52	0.02	0.06	1
<i>Canthidium</i> sp.1	N	*	0.26	0.05	48	0.16	0.05	13
<i>Canthon</i> aff. <i>dives</i>	N	*	0.76	0.16	49	0.24	0.07	7
<i>Canthon</i> aff. <i>unicolor</i>	N	**	0.48	0.11	56	0.07	0.03	3
<i>Canthon virens</i>	N	**	9.21	1.81	78	0.92	0.19	6
<i>Canthon lamproderes</i>	N	***	1.44	0.48	56	0.02	0.01	0
<i>Coprophanæus spitzii</i>	N	*	0.55	0.12	52	0.21	0.05	13
<i>Deltochilum elevatum</i>	N	***	0.26	0.05	51	0.03	0.01	2
<i>Dichotomius semiaeneus</i>	N	*	0.25	0.16	28	0.08	0.01	0
<i>Generidium cryptops</i>	N	*	0.32	0.13	40	0.02	0.01	1
<i>Oxysternon paleo</i>	N	*	0.47	0.11	74	0.11	0.04	7
<i>Phanaeus palaeno</i>	N	*	0.82	0.15	61	0.68	0.08	15
<i>Canthidium</i> aff. <i>breve</i>	I	*	1.22	0.32	12	4.08	0.63	66
<i>Canthon chalybaeus</i>	I	*	0	0	0	0.67	0.19	62
<i>Dichotomius</i> aff. <i>ascanius</i>	I	*	0.03	0.02	2	0.18	0.04	52

Table S1. Table of mean abundance per pasture, significance level of abundance (\* $P < 0.05$ , \*\* $P < 0.005$ , \*\*\*  $P < 0.001$ ) between pasture systems according to Kruskal-Wallis test, and mean dry body weight per species (see Methods) of dung beetles collected and their functional guild in native and exotic pastures in Carrancas, Minas Gerais, Brazil.

Tribe/ Species	Mean abundance		$\chi^2$	Mean body weight (g)
	Native	Introduced		
<b>Ateuchini</b>				
<i>Anomiopus</i> aff. <i>nigrocoerulus</i>	0	0.09	1.32	0.0055
<i>Anomiopus</i> sp.1	0	0.04	0.66	0.0060
<i>Ateuchus</i> aff. <i>puncticolis</i>	0.14	0.19	0.1	0.0148
<i>Ateuchus striatulus</i>	0.14	0.33	0.37	0.0104
<i>Ateuchus subquadratus</i>	0	0.04	0.66	0.0320
<i>Ateuchus vividus</i>	0.5	0.09	4.3*	0.0198
<i>Canthidium</i> (C.) sp.1	1.57	1	5.36*	0.0175
<i>Canthidium</i> aff. <i>breve</i>	7.35	24.71	22.85***	0.0077
<i>Canthidium</i> aff. <i>humerale</i>	2.07	0	6.13*	0.0044
<i>Canthidium barbaticum</i>	18	5.95	35.02***	0.0063
<i>Canthidium decoratum</i>	45	10.95	52.99***	0.0216
<i>Canthidium depressum</i>	0.35	0	1.51	0.0050
<i>Canthidium marseuli</i>	1.5	0.14	15.73***	0.0230
<i>Eutrichilum hirsutum</i>	1.57	0.42	3.86*	0.0015
<i>Eutrichilum</i> sp.1	0.14	0	3.03	0.0010
<i>Generidium bidens</i>	0.64	0.04	2.11	0.0010
<i>Generidium cryptops</i>	1.92	0.14	12.64***	0.0039
<i>Scatonomus thalassinus</i>	0.14	0	3.03	0.0435
<i>Trichillum adjunctum</i>	20.78	6.76	6.06*	0.0030
<i>Trichilum extenepuctatum</i>	1.50	1.28	0.27	0.0010
<i>Trichilum heydeni</i>	0.07	0	1.51	0.0010
<i>Uroxys</i> sp.1	0.21	0.28	0.72	0.0013
<b>Canthonini</b>				
<i>Agamopus unguicularis</i>	5.14	1.66	8.80**	0.0018
<i>Agamopus viridis</i>	2.21	0.33	23.30***	0.0010
<i>Canthon</i> ( <i>Glaphyrocanthon</i> ) sp.	0	0.04	0.66	0.0060
<i>Canthon</i> aff. <i>bispinus</i>	0	0.33	0.32	0.0066
<i>Canthon</i> aff. <i>dives</i>	4.57	1.47	10.94***	0.0481
<i>Canthon</i> aff. <i>janthinus</i>	1.28	0	9.29**	0.0027
<i>Canthon</i> aff. <i>podagricus</i>	0	0.04	0.66	0.0050
<i>Canthon</i> aff. <i>pseudoforcipatus</i>	0	0.04	0.66	0.0020

**Continuation**

<i>Canthon aff. unicolor</i>	2.92	0.42	20.38***	0.0456
<i>Canthon virens</i>	55.28	5.61	27.79***	0.0282
<i>Canthon chalybaeus</i>	0	4.04	23.73***	0.0207
<i>Canthon histrio</i>	0.07	0.38	2.58	0.0413
<i>Canthon lamproderes</i>	8.64	0.14	29.80***	0.0623
<i>Canthon lituratus</i>	0	0.38	4.76*	0.0060
<i>Canthon muticus</i>	0.35	0	6.13*	0.0072
<i>Canthon ornatus</i>	0.21	0	4.57*	0.0230
<i>Canthon quadripunctatus</i>	0.07	0	1.51	0.0210
<i>Canthonella sp.</i>	0.57	0.19	1.81	0.0010
<i>Deltochilum aff. aureopilosum</i>	3	1.47	0.09	0.0726
<i>Deltochilum dentipes</i>	0	0.04	0.66	0.0499
<i>Deltochilum elevatum</i>	1.57	0.19	18.26***	0.2396
<i>Deltochilum pseudoicarus</i>	0.21	0.14	0.26	0.5406
<i>Pseudocanthon xanturus</i>	1.21	0.19	6.48*	0.0011
<i>Sylvicanthon foveiventris</i>	0	0.04	0.66	0.0180

**Coprini**

<i>Dichotomius aff. ascanius</i>	0.21	1.09	7.11*	0.1719
<i>Dichotomius bos</i>	0.71	0.76	0.0126	0.3234
<i>Dichotomius carbonarius</i>	0.07	0.14	0.05	0.1502
<i>Dichotomius luctuosus</i>	0	0.42	2.00	0.1552
<i>Dichotomius mórmon</i>	0	0.04	0.66	0.5820
<i>Dichotomius nisus</i>	0	0.04	0.66	0.2560
<i>Dichotomius semiaeni</i>	1.50	0.04	4.91*	0.0836
<i>Isocoprins inhatius</i>	0	0.23	3.37	0.9254
<i>Ontherus digitatus</i>	0.50	0.28	1.71	0.0240
<i>Ontherus ulcopygus</i>	0.50	0	6.13*	0.0673

**Eurysternini**

<i>Eurysternus parallelus</i>	0.14	0.14	0.001	0.0416
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**Continuation****Onthophagini**

<i>Onthophagus aff. ranunculus</i>	4.71	3.28	2.17	0.0099
<i>Onthophagus bucculus</i>	6.92	3.95	0.7	0.0065

**Phanaeini**

<i>Coprophanaeus horus</i>	3.07	3.09	0.55	0.3035
<i>Coprophanaeus magnoi</i>	0	0.23	2.00	0.5308
<i>Coprophanaeus spitzii</i>	3.35	1.28	6.97*	0.5661
<i>Dendropaemon aff. smaragdinum</i>	0.07	0	1.55	0.0180
<i>Oxysternon palemo</i>	2.85	0.71	14.61***	0.1861
<i>Phanaeus kirby</i>	3.64	0.80	9.25**	0.2366
<i>Phanaeus palaeno</i>	4.92	2.04	14.80**	0.1564

FIGURE 1. (A) Map of study area showing Carrancas city in the South of Minas Gerais (MG) State in SE Brazil. (B) The seven farms sampled are represented by black dots in the city limits. (C) The typical sample design for each of the 35 studied pastures distributed in the farms.

FIGURE 2. Individual-based species accumulation curves for dung beetle communities within different pasture systems. The dotted lines are 95% CI, illustrating that there was no significant difference between native and exotic pastures.

FIGURE 3. Observed mean richness and abundance of dung beetles (per pasture) in native (n=14) and exotic (n=21) pastures (\*p<0.05, \*\*p< 0.005) based on Poisson generalized linear model (GLM).

FIGURE 4. Rank-abundance distribution of dung beetles species in native and exotic pastures in the Carrancas's farms in an agricultural landscape.

FIGURA 5. Non-metric multidimensional scaling (NMDS) ordination based on a distance matrix computed with Bray-Curtis similarity index between pasture systems: native pasture and exotic pasture. NMDS (A) shows the difference in community composition (presence/absence species data) and NMDS (B) shows the difference based on community structure (abundance of individuals).

Fig. 1

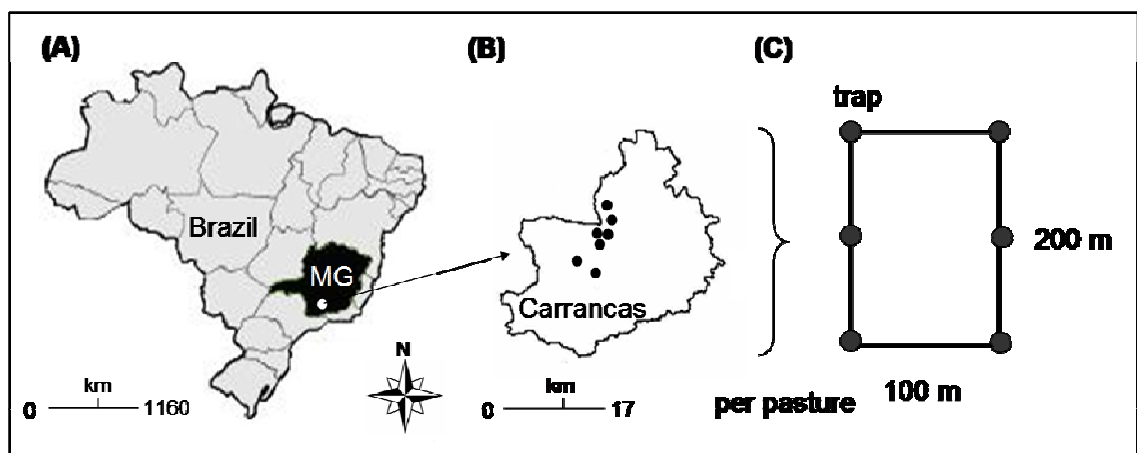


Fig. 2

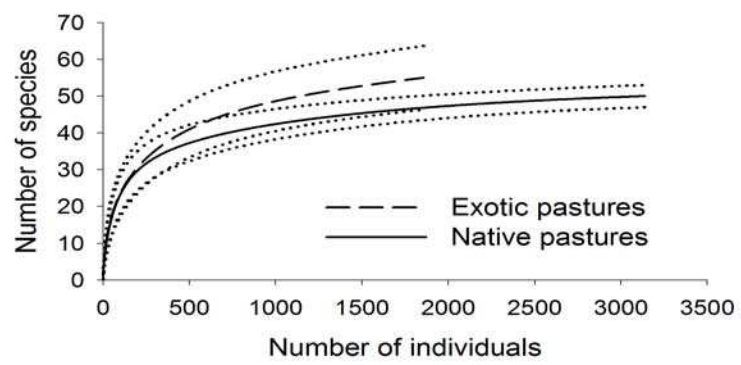


Fig.3

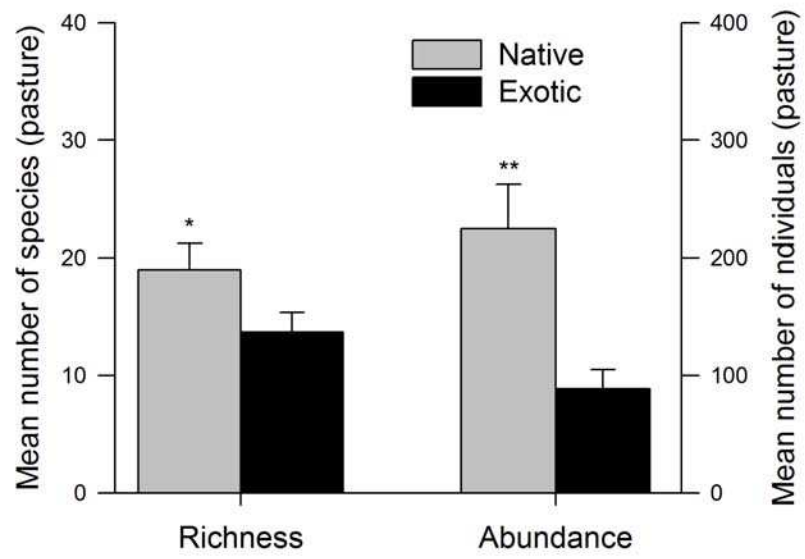
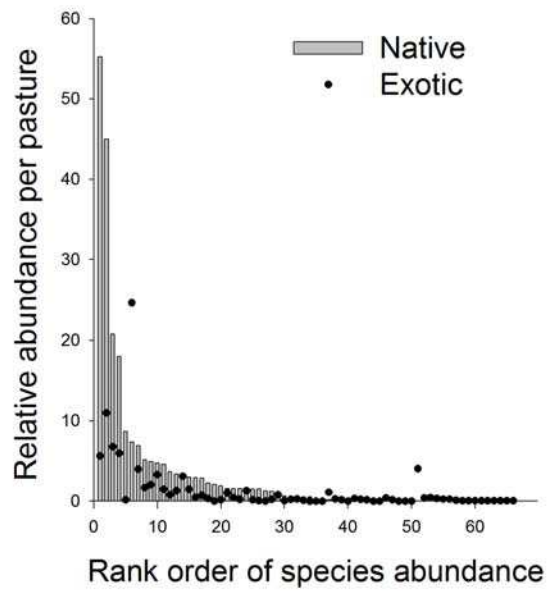


Fig.4





## CAPÍTULO 2

**Ecological drivers of dung beetles in the Cerrado grasslands: does introduction of exotic pastures alter community-environment relationships?**

## Summary

Brazilian savanna (Cerrado) has been threatened by increasing economic pressures for cattle grazing; either on native grasslands (*campo limpo*) managed with fire for pasture use, or on introduced pastures, where exotic grasses replaced the native grasslands. We evaluate the environmental drivers that could affect the dung beetle community and examine if the introduction of exotic pastures can affect those drivers. We considered local drivers as sand proportion in soil, soil penetrability and time since last disturbance (fire or implantation), and landscape drivers as distance to nearest forest and nearest different pasture system. We compared environmental drivers for both pasture systems using multiple model approach. We collected 4996 individuals of 66 species. For native and introduced pastures, species richness was not affected by any of our environmental variables in multiple model approach (AICc). Dung beetle abundance in native pastures decreased with soil penetrability and increased with distance to nearest forest and sand proportion. Time since last fire management and nearest introduced pasture had weak and negative effects on abundance. Beetle abundance in introduced pastures increased with distance to nearest forest and decreased with soil penetrability, sand proportion and with distance to nearest native pastures. Time since introduction had a weak and negative effect on abundance. Our environmental variables did not alter species composition within pasture systems. We interpreted that, for both pasture systems, richness was driven by regional species pool. Sand proportion, soil penetrability, surrounding habitat and time since disturbance were determinants of dung beetle abundance in both pastures systems. However, the introduction of exotic pastures changed the effects of the environmental drivers on dung beetle communities, probably through soil management practices. We showed that habitat replacement goes beyond changes in vegetation cover: exotic grass introduction changes community-environment relationship for dung beetles.

**KEYWORDS:** Brazilian savanna, fire, native grassland, landscape, soil, Scarabaeinae, *Urochloa*.

## **Introduction**

The Brazilian savanna (Cerrado) is a tropical ecosystem composed by a mosaic of grassland and forested vegetation (Ratter et al., 1997). It is the second largest biome in Brazil and it is considered to be one of the most biodiversity-rich and threatened ecosystem in the world (Myers et al., 2000, Klink & Machado, 2005). The main threats are the replacement of native habitats by monocultures (e.g. exotic pastures for cattle, sugarcane for ethanol, and soybeans) and the increased burning frequencies, brought about by changes in land-use and fire management (Silva et al., 2006, Scharlemann & Laurance, 2008, Queiroz, 2009, Bond & Parr, 2010).

The loss of native Cerrado plant biodiversity due to erroneous fire management and due to native to exotic monocultures replacement is obvious and well studied (e.g. Ratter et al., 1997, Pivello et al., 1999, Mistry, 1998, Pivello, 2006, Silva et al., 2006). However, there is still a lack of information about the impact of these factors on the inhabiting fauna, especially for insects. Insect communities are useful tools for evaluating anthropogenic impacts in tropical ecosystems, mainly due to their rapid response to environmental changes and an easily sampling effort (e.g. DeSouza et al., 2003; Carrijo et al., 2008; Vasconcelos et al., 2009, Louzada et al., 2010). Among the insects, dung beetles (Coleoptera: Scarabaeidae: Scarabaeinae) can be used as cost-effective indicators of anthropogenic disturbance (Halfpter & Favila, 1993), and can detect changes in soil characteristics due to their foraging and nesting habits (Hanski & Cambefort, 1991), above-ground disturbance (e.g. vegetation replacement and fire), and changes in the composition of the landscape (Navarrete & Halfpter, 2008; Almeida & Louzada, 2009; Louzada et al., 2010).

Changes in dung beetle community between native and introduced pastures in Cerrado were detected by Almeida et al. (in press). However, there is a poor understanding about the mechanisms that drive these changes. As habitat modification and replacement can alter community-environment relationships in tropical forests (Didham et al., 1998, Silveira et al., 2010), we were interested in examining how the environmental drivers of dung beetle communities in Cerrado biome. We evaluated the environmental drivers that could affect the dung beetle community and examine if the introduction of exotic pastures can affect those drivers. We considered local drivers as sand proportion in soil, soil penetrability and time since last disturbance (fire or implantation), and landscape drivers as distance to nearest forest and nearest different pasture system.

First, we provide a baseline understanding the community-environment relationships in native Cerrado using fire management, landscape composition, and soil characteristics. We then use a similar suite of environmental variables to examine these relationships on the dung beetle community in exotic pastures.

## **Material and Method**

### **Study site**

The study was conducted in Carrancas, in the south of Minas Gerais State, southeastern Brazil (21°28'24" S, 44°39'05"W), a region of transition of Cerrado and Atlantic forest biomes (Oliveira Filho et al., 2004). The altitude varies between 900m and 1200m. The climate is Cwa (Koppen classification- humid subtropical, with hot, humid summers and cold winters). The region has approximately 1480mm mean annual precipitation, with a mean annual temperature of 15° C (Oliveira-Fillho et al., 2004). The forests present in the areas were riparian forests with elements of Atlantic forest: a semi-deciduous forest (Oliveira-Fillho et al., 2004).

The south region of Minas Gerais is one of the most important milk producers in Brazil (Zoccal et al., 2006) and the majority of the producers are smallholders; like in Carrancas that have the milk production to dairy factories as main economical activity (IBGE, 2006) and part of the traditional "Fine Cheese Circuit" in Brazil (Leandro, 2008).

Traditionally, the smallholders use *campo limpo* (native Cerrado grasslands) as extensive pastures to grazing the cattle and they must burn the area each two years (Fontanelie & Jacques, 1988, Jacques, 2003). Native grasslands are composed of several native species of grass (Poaceae) and other plant families, including dicotyledon plants (Carvalho, 1993, Rodrigues & Carvalho, 2001, Munhoz & Felfili, 2006)

Around 30 years ago, Brazilian government has been providing incentives to modernization of agricultural lands (Silva, 2000), including the replacement of native pastures into introduced pastures (personal contact, EMATER- Company of technical support and rural extension-Carrancas) due to the higher carrying support of exotic grass (Martha Junior & Vilela, 2002) and as alternative to negate fire management in native pastures.

The introduced pastures in the studied region consist in monocultures of the African grass *Urochloa* spp. (*Urochloa* P. Beauv. spp. (= *Brachiaria* (Trin.) Griseb. spp.), commonly used due to its resistance to low fertility and acid soils, characteristics of Cerrado (Martha Junior & Vilela, 2002).

### **Experimental design**

We sampled 35 pastures (sites), at least 300 m apart of each other, in dairy farms in Carrancas. The pastures consisted in 14 native grasslands used as pastures and with different time since last disturbance (fire management- LF), varying between three months and 36 months, and 21 introduced pastures with the African grass with, different time since last disturbance (time of exotic grass implantation-TI), varying between 12 months and 360 months.

### **Dung beetle sampling**

Dung beetles were sampled using pitfall traps (19cm diameter, 11cm depth) buried flush with the ground and filled with 150ml of saline solution and detergent. The traps were baited with human faeces because it is the most attractive types of dung to most species of dung beetles (Howden & Nealis, 1975, Larsen & Forsith, 1995).

The trap has a base made of wire in the form of a hoop to accommodate a small plastic container (diameter 4 cm, height 4 cm) where the bait was placed. The based was fixed in the soil in a way that the bait container was sustained in the centre of the trap. We also used a small plastic cover (20 cm diameter) sustained by three sticks to protect the trap from rain. Six traps were placed at each pasture, separated by 100 m in a rectangle design. We had an amount of 210 traps in the study (for the same sampling design see Almeida et al. 2010, in press). Trapping was conducted in a 48 h period at each pasture during January 2008, the rainy season, best period of the year to sample dung beetles in the tropics (Martínez & Vásquez, 1995, Milhomem et al., 2003).

Dung beetles were identified until genus and species when possible, using the reference collection of Invertebrate Ecology and Conservation Laboratory (IEC) at Universidade Federal de Lavras, Brazil and with the help of the specialist Dr. Fernando Vaz-de-Mello. Voucher specimens were placed at the IEC and collection of Universidade Federal do Mato Grosso (UFMT).

### **Environmental variables**

Dung beetles are intimately related with soil due to their habits of digging the soil for nesting and shelter (Hanki & Cambefort, 1991). Soil samples resulted of the trap buried was collected for laboratorial analyses of texture. The texture analysis verified the proportion of sand, silt and clay in the samples and it was conducted in Department of Soil Sciences in the Universidade Federal de Lavras (UFLA).

In order to obtain a degree of soil penetrability (SPN), or a biological measure of how dung beetles could be benefited digging soft soils, we obtained SPN data using a penetrometer in each trap point. The penetrometer used was a soil impact penetrometer, Stolf model (Stolf, 1991) which measure is obtained through the impact of a known weight falling from a certain height in free fall on a stem, making the stem penetrates in the soil, generating the SPN, measured in centimetres.

To evaluate the influence of the surrounding pastures habitats, the distance of each trap to the next different habitat was measured. In native pastures, we measured distance to nearest forest (NF) and distance from nearest introduced (NI) pasture. In introduced pastures, we measured the distance to nearest forest (NF) and distance to nearest native (NN) pasture. All the distance measures were obtained with a GPS Garmim, model 76CSx, and confirmed with a satellite images from Google Earth, because images made with GIS technology did not detect differences between native and introduced grasslands (Brannstrom et al., 2008).

In order to evaluate the influence of habitat disturbance on both pasture systems, we used as explanatory variables, time after last fire, for native systems, and time after pasture implantation, for introduced systems.

### **Statistical analysis**

In order to avoid using collinear explanatory variables in the same models (Zuur et al., 2010), we evaluated the correlation of the variables related to soil texture (sand, silt, clay), and SPN in each pasture system with non-parametric Spearman's rank correlation test (Crawley, 2007). We considered strongly correlated those variables which had correlation ( $r_s$ ) higher than 0.70 and  $P < 0.05$  of significance. As a result we chose sand proportion and soil penetrability to represent the soil variables, because these presented no strong correlation in the native pastures (Table 1).

We tested the hypotheses that the environmental drivers of dung beetle communities were time since last disturbance, surrounding habitat and soil characteristics. To test these hypotheses, we used as explanatory variables LF, NF, NI, S, SPN for native pastures. Thereafter, to evaluate if the introduction of exotic grass altered the community-environment relationship, we used TI, NF, NN, S and SPN as explanatory variables to adjust the models with the introduced pastures' data.

We used the multimodel inference (Burnham & Anderson, 2002) that is a information-theoretic approach, that compares and ranks models using Akaike's Information Criterion corrected for small sample size (AICc) to evaluate the explanation

provided by each adjusted model (Burnham & Anderson, 2002). We used the “dredge” function from the “MuMIn” package (Bartón, 2009) to test models defined by all possible variable combinations, and rank them by their AICc-based model weight ( $\omega$ ). This statistic provides the relative weight of each particular model, varying from 0 (no support) to 1 (complete support), relative to the entire model set. All models with  $\Delta\text{AICc} < 2$  were considered with substantial support, (Burnham & Anderson, 2002).

In order to evaluate the effects of environmental variables on dung beetle species composition within each pasture system, we used BIOENV analysis, so as to select the best subset of environmental variables with maximum (rank) correlation with community dissimilarities (Clarke & Warwick, 2001). The community data abundance matrix (log-transformed) per pasture system, was tested against environmental parameters matrix (constructed utilizing normalized Euclidian distances). The matrices were statistically compared by Spearman’s rank correlation test. BIOENV analysis examines all possible combinations of environmental variables that best account for the observed species distribution. Each environmental variable was analysed separately, then in pairs, triplets, up to all at the same time, and the correlation coefficients of each combination of explanatory variables was calculated. We chose the combination with the best correlation coefficient for each explanatory variable. The BIOENV analysis was conducted with Primer v. 5 (Clarke & Warwick, 2001).

## **Results**

We collected 4996 individuals of 66 dung beetles species. The species were distributed in six tribes: Ateuchini, Canthonini, Coprini, Eurysternini, Onthophagini and Phanaeini. We found 3139 individuals of 50 dung beetles species in native pastures and 1857 individuals of 55 species in introduced pastures (see Almeida et al., 2010, in press). We summarize the results in Table 2, so as to facilitate comparisons between native and introduced pastures.

### **Native pastures**

The multiple model approach revealed a weak influence of all environmental variables on dung beetles species richness (best ranked model,  $\omega=0.112$ , Table 3). For number of individuals, the best ranked model in multiple model selection revealed support for the positive influence of sand proportion and nearest forest, and a negative influence of soil penetrability ( $\omega= 0.337$ , Table 3). However, the second and third ranked models that include the variables distance to nearest introduced and time since

last fire should also be considered similar (Table 3) because they grouped small  $\Delta AICc$  and small evidence ratios (Burnham & Anderson, 2002). Dung beetle species composition was not correlated with any of our explanatory variables (S, SPN, LF, NI, NF) within native pastures according to BIOENV correlation test (Table 4).

### **Introduced pastures**

In order to compare if introduced pastures had the same environmental drivers as native pastures, we used the same statistical approach used in native pastures, but exchanged the disturbance: time since fire for time since implantation. The multiple model approach revealed that there was no relationship between our explanatory variables and dung beetle species richness (Table 5). The best ranked model had a low weight of just  $\omega = 0.098$  (Table 5). For abundance, the best model ranked (model 1, Table 5) included the negative influence of the proportion of sand, soil penetrability and distance to nearest forest, and the positive influence of distance to nearest native pasture. However, the second model was also given strong support, suggesting the negative effect of time since implantation may also be important (Table 5). Dung beetle species composition was not influenced by our environmental variables (S, SPN, NN, NF, TI), and the best correlation (with sand proportion and time since exotic grass implantation) was very weak ( $R=0.19$ ; Table 4).

### **Discussion**

The consequences of replacing native grasslands pastures by monocultures of exotic grass can go beyond the plant community and their herbivores. First, we discuss our results investigating the environmental drivers of dung beetle communities in native grasslands, and examine whether time since last disturbance (fire), surrounding habitats (introduced pastures and forests) and local soil characteristics (sand proportion and soil penetrability) affect the dung beetle community parameters. Second, we discuss whether similar environmental variables affect the community in introduced pastures, and discuss how the introduction of exotic grass alters community-environment relationships.

### **Dung beetle species richness**

Although restricted to a unique geographical region, the non-significance of environmental variables on the number of species suggests that the regional pool of species contributes more to dung beetle's richness than our environmental variables for

both pasture system. The permeability of open vegetation areas probably reflect the absence of barriers to dispersal, so that all dung beetle species in the region may have a chance of arriving at any trap, whatever pasture system it is located. For example, Hanski and Cambefort (1991) showed that dung beetle species of open areas can disperse long distances to explore ephemeral abundant resources. Several authors suggest that local dung beetle's communities are conditioned by vegetation structure of surrounding landscape and the connectivity between habitats (Roslin & Koivunen, 2001, Navarrete & Halffter, 2008, Numa et al., 2009). Cerrado is a predominantly open landscape, enabling unhindered movement of flying insects, which provides high connectivity when compared with forest dominated landscapes. Our results corroborate the study of Almeida and Louzada (2009) that found no differences in dung beetles' diversity in native Cerrado open areas. Therefore, provided food resource is available, dung beetles can use introduced pastures as a transient habitat.

### **Dung beetle community composition**

Community composition was not influenced by any measured environmental variables, suggesting that in grassland landscapes, high connectivity both in local and in larger scale environmental effects upon dung beetle communities is the main driver of species distribution within native and introduced pastures as well as to species richness. This explains the homogeneity of species composition between pasture systems: although exotic grass introduction alters local soil characteristics, and their effect upon dung beetle abundance, these changes are not sufficient to affect species composition, because of their high mobility.

The negative influence of distance to nearest forest on beetle abundance, in both pasture systems, indicates that forests may act as a barrier to the dispersion of dung beetles. Dung beetle species from open areas (introduced pastures or native Cerrado grasslands) rarely penetrate Cerrado forests, and forest species do not penetrate open areas (Spector & Ayzama, 2003; Navarrete & Halffter, 2008; Almeida & Louzada, 2009). Of the 66 dung beetle species that we found in pastures, only seven from 2390 individuals collected by Renan Macedo (UFLA, unpublished data), and only five from 2363 individuals collected by Almeida & Louzada (2009), were also found in forest habitat of the same region of Carrancas. This shows that Cerrado forests may hinder the movement of open-area dung beetle species, both as a mechanical barrier, and as a biological, low connected, habitat.

Indeed, Spector & Ayzama (2003) found a sudden species composition change, when studying dung beetle assemblage along a savanna-forest ecotone, and Halffter & Arellano (2002) verified that forest-species rarely expand their habitats to areas without a closed canopy.

### **Dung beetle abundance**

Sand proportion and soil penetrability were the most important local factors driving dung beetle abundance; these effects had strong support in the multiple model approach. In other tropical savannas, in western Africa, most dung beetles prefer soft and sandy soil (Hanski & Cambefort, 1991), while *Onitis* species from Australia savannas are often found nesting in moist sandy soil (Edwards & Aschenborn, 1987). Therefore, the dependence of dung beetles on sandy soil may be characteristic of savanna habitats. In this way, we can consider the Cerrado native dung beetles more dependent of high sand proportion and low soil penetrability or softness to their nesting activities in native pastures. Probably, these characteristic are related with nest stability. Different species of dung beetles are able to construct several connected nesting chamber in the soil and the resistance of the soil could be important to the stability of the tunnels. Besides, dung beetles mix the brood balls with soil because the moisturizer must be controlled and sand retains less water than clay (Edwards & Aschenborn, 1987). However, further studies must be conducted to test these hypotheses.

The local effects of sand proportion and soil penetrability were also important to introduced pastures as in native pastures. But the effect of sand proportion was the opposite: less sand more individuals. Moreover, the time of implantation must be considered and could be barely separated of the soil characteristics effects in introduced pastures. The implantation of monocultures of exotic grass in Cerrado can affect soil properties. Normally the introduction of exotic grass in Cerrado took place in native grasslands. The drastic implantation process consists in (1) remove the native grassland and use the plough to turn soil over, (2) add lime to alter the soil pH (the soil is acid in Cerrado landscape), (3) add fertilizers and buy the seeds to grow the exotic grass (Martins et al., 2004).

The mechanical stress (the act to plough) and liming the soil can destabilizes the soil structure (Westerhof et al., 1999, Mendes et al., 2003) that could be important to nesting habits of dung beetles as we commented above. The study of Gomes et al. (2004) found that the proportion of sand in soil can contribute with more or less proportion of soil stability depending of the soil classification. In that case, we can

assume, for native pastures never limed and ploughed, that the proportion of sand is better to dung beetle's nest stabilization. Less sand could contribute to a destabilization of nests tunnels accompanied of higher soil penetrability: harder soils can support more individuals. In this way, the community-environment relationship changed in introduced pastures when compared with native pastures.

Although species richness was not affected by our environmental variables, they had a greater effect on abundance. The number of individuals was strongly affected by the surrounding habitat. Native pastures near to forest and far from introduced pastures had fewer individuals. Again, an evidence of the connectivity of open areas (even introduced) that favoured the dispersion of dung beetles. However, for introduced pastures, the negative effect of distance to nearest native pasture on dung beetle abundance can be leading us to interpret native pastures as the source of native dung beetles to introduced pastures. On the other hand, forests can be considered a biological barrier for individuals for both pasture systems. The few individuals from forests that manage to disperse to other habitats looking for food resource can be considered "tourists" (Fagan et al., 1999, Dangerfield et al., 2003). Long distance to nearest forest means a higher amount of open areas (source of individuals) and more permeable landscape to dung beetles activities. Even with the implantation of exotic pastures in the region, the matrix of our study sites still has open native Cerrado vegetation (grasslands and *campo sujo*) as the predominant land-cover (IBGE, 2006, Scolforo et al., 2008) and forests were associated with areas nearby streams (Oliveira-Filho et al., 2004).

Probably due to its distribution, forests in Carrancas were normally distributed in soils with lower sand proportion (Oliveira-Filho et al., 2004) when compared with other Cerrado's vegetation types (Almeida, 2006). Indeed, we found an increase of sand proportion in native pastures far from forests.

The amount of time since the last fire event was also hypothesised to be an environmental driver of the community metrics we assessed. Despite the fact that time since fire disturbance appeared just in the third model in multiple model selection, the model has a small difference with first and second model and therefore should be given some support since Cerrado is considered fire-dependent (Pivello, 2006).

The abundance had a decrease with recent fire events, however, it was expected species resilience to fire in Cerrado due to its evolutionary history and we found the same resilience in studies with ants in African savanna (Parr et al., 2004), termites in burned Cerrado areas (DeSouza, 2003) and for dung beetle of Amazonian savanna (known as disjunct Cerrado), which effect of fire was completely indirect (Louzada et

al., 2010). Besides, the individual recolonization leading to a relative fauna quick recover (few months) was found for soil arthropods (Pais & Varanda, 2010) and leaf-miner (Marini-Filho, 2000). Our study was conducted after three months of disturb and the peak of the sprouting of new leaves vegetation which can attract more herbivores insects (Vieira et al., 1996), and native herbivores mammals (Pivello, 2006) and even the cattle, that provided food resource for dung beetles. We therefore assume that fire is a natural component of Cerrado and dung beetles are resilient and able to recolonize areas of burned Cerrado after a short-time period.

### **Community-environment relationship**

The general picture that emerges from our analyses was the contrast of the environmental drivers between native and introduced pastures. The habitat replacement changed the community-environment relationship in introduced pastures due to drastic soil management. Even though the species richness were driven by the same pool of regional species, local effects had opposite influence on number of individuals comparing introduced and native pastures. Sand proportion was probably altered by the implantation that also had a negative influence on dung beetle abundance by itself. The proximity with native pastures probably contributes with dung beetles that can disperse and use the introduced pastures as transient habitat.

### **Conclusion and conservation implications**

The introduction of exotic plants is a worldwide problem that is responsible for drastic changes in natural ecosystems, primarily the loss of native plant species (Brooks et al., 2008) and the cascade effect of its implantation as changes in fire regimes (Ramos-Neto & Pivello, 2000), available food to native fauna (Trolle et al., 2007) and soil compaction due to higher reckoned carrying support (Meirelles et al., 2004) when compared with native pastures. The multiple statistical approach detected more subtle effects (the time since last disturbances in both pasture systems) that contributes more with secondary effects- the cascade effects. The time since implantation affecting negatively the number of individuals helped us with the comprehension of habitat replacement goes beyond changes in vegetation cover: exotic grass introduction change community-environment relationship for dung beetles especially due to soil management.

The time since last fire management had a negative but weak effect on dung community. Normally, the conscious management made by farm producers involves a

mild fire in native pastures each two years and just in the end or the beginning of the wet season (Evangelista et al., 1993). Of course, even though a fire-adapted biome as Cerrado, the high frequency and intensity of fire can definitely destroy the native vegetation and fauna (Klink & Machado, 2005). The procedures to make fire management are regulated by Minas Gerais law (Minas Gerais, 2006). The producers must construct fire breakers to control the burned area (Pivello, 2006) and it was observed in Carrancas region (Almeida- personal observation). Besides, dung beetles could stay buried in soil and the offspring could be safe, also buried in the nest the whole period of maturation. The mild fire is also recommended in some native areas to prevent wild fire in some natural reserves in Brazil (Ramos-Neto & Pivello, 2000, Pivello, 2006). Also, the time since last fire management did not have influence on dung beetle composition within native pastures probably for the same reason. Almeida et al., (in press) showed that just pasture system can contribute to differences in dung beetle species composition.

Finally, we can conclude that Cerrado native vegetation is a source of dung beetle species. The native habitat loss generates a cascade effect that changes the vegetation, the natural fire-regime, the soil properties and the surrounding areas; affecting in a direct way the dung beetle community and the whole Cerrado landscape. Socio-economic issues were still rarely taken into account the importance of species diversity and the possibility of both agro-environments and biodiversity being economical available (Poschlod et al., 2005). We suggest that the discussion between researchers and farmers can be an important agent of changes in farms' management that take in account a balance between socio-economic and environmental issues.

### **Acknowledgments**

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Table 1. Summary of Spearman's correlation test showing the correlation ( $r_s$ ) among the soil variables: soil penetrability (SPN), silt, clay and sand in both pasture system (native and introduced). (\*) means the significance of test ( $P<0.05$ ), (\*\*) means the significance of test ( $P<0.01$ ) and (\*\*\*) means the significance of test ( $P<0.001$ ). Bold variables were chosen as explanatory variables, and variables were considered collinear when  $r_s>0.70$ .

Native grassland		Soil variables		
	Silt	Clay	<b>Sand</b>	
<b>SPN</b>	0.40	0.57 (*)	-0.61 (*)	
Silt		0.69 (**)	-0.88 (***)	
Clay			-0.92 (***)	
Introduced pasture		Soil variables		
	Silt	Clay	<b>Sand</b>	
<b>SPN</b>	0.01	-0.007	-0.01	
Silt		0.45 (*)	-0.72 (***)	
Clay			-0.90 (***)	

Table 2. Summary of results using multiple model information-theoretic approach using Akaike's Information Criterion corrected for small sample size (AICc). The results of BIOENV species composition analyses were also summarized. BIOENV tested the best subset of environmental variables correlated with dung beetle community dissimilarities. The symbols (+) and (-) mean the influence of the variable to species richness and abundance in each pasture system (native and introduced); (ns) means not significant variable; (us) unsupported.

Variables	Richness	Abundance	Composition
	<b>Native</b>		
Sand proportion	us	+	us
Nearest forest	us	+	us
Nearest introduced	us	-	us
Soil Penetrability	us	-	us
Last fire	us	us	us
<b>Introduced</b>			
Sand proportion	us	-	us
Nearest forest	us	+	us
Nearest native	us	-	us
Soil penetrability	us	-	us
Time of implantation	us	-	us

Table 3. AICc-based model selection for (i) dung beetle species richness, and (ii) abundance in **native pastures**. Generalized linear mixed-effect models used “pasture” as a random factor and include Sand Proportion (S), Soil Penetrability (SPN), Time since Last Fire (LF), Distance from Nearest Forest (NF), and Distance from Nearest Introduce Pasture (NI) as fixed factors. We also show the number of model parameters (K), AICc differences ( $\Delta$ ) and Akaike weights ( $\omega$ ). Models are shown up top 95% of cumulative Akaike weights (Cumulative  $\omega$ ) or the intercept (no variables). The symbol ( $^+$ ) indicates positive relationship and ( $^-$ ) negative relationship with the variables abundance and richness.

Models rank	Model	K	AICc	$\Delta$	$\omega$	Cumulative $\omega$
<i>(i) Richness</i>						
1	SPN $^-$	4	119.9	0.000	0.112	0.112
2	NF $^+$	4	120.6	0.656	0.081	0.193
3	SPN $^-$ +NF $^+$	5	120.8	0.895	0.071	0.264
4	S $^+$ +SPN $^-$	5	121.0	1.048	0.066	0.330
5	<i>Intercept</i>	3	121.3	1.403	0.055	0.385
Models rank	Model	K	AICc	$\Delta$	$\omega$	Cumulative $\omega$
<i>(ii) Abundance</i>						
1	S $^+$ +SPN $^-$ +NF $^+$	6	731.6	0.000	0.337	0.337
2	S $^+$ +NI $^-$ +SPN $^-$ +NF $^+$	7	731.9	0.336	0.285	0.622
3	S $^+$ +SPN $^-$ +NF $^+$ +LF $^-$	7	733.0	1.414	0.166	0.788
4	S $^+$ +NI $^-$ +SPN $^-$ +NF $^+$ +LF $^-$	8	733.3	1.724	0.142	0.930
5	S $^+$ +SPN $^-$	5	737.0	5.372	0.023	0.953

Table 4. Results of BIOENV analyses comparing best correlation (R) models of dung beetle's species composition in both pasture system and environmental variables: time since last fire (LF), sand proportion in soil(S), distance to nearest introduced pasture (NI), distance to nearest forest (NF), time of exotic grass implantation (TI), distance to nearest native pasture (NN) and "soil penetrability" (SPN).

BIOENV			
Pasture system	Model size	Best Models	Correlation (R)
<b>Native</b>	1	LF	0.03
	2	LF+SPN	0.06
	3	LF+S+SPN	0.02
	4	LF+S+NF+SPN	-0.08
	5	LF+S+NF+NI+SPN	-0.11
<b>Introduced</b>	1	TI	0.13
	2	TI+S	0.19
	3	TI+S+NN	0.14
	4	TI+SPN+S+NN	0.11
	5	TI+S+SPN+NN+NF	0.09

Table 5. AICc-based model selection for (i) dung beetle species richness, and (ii) abundance of individuals in **introduced pastures**. Generalized linear mixed-effect models used pasture as a random factor, and include Sand Proportion (S), Soil Penetrability (SPN), Time of Introduction (TI), Distance from Nearest Forest (NF), and Distance from Nearest Native Pasture (NN) as fixed factors. We also show the number of model parameters (K), AICc differences ( $\Delta$ ) and Akaike weights ( $\omega$ ). Models are shown up top 95% of cumulative Akaike weights (Cumulative  $\omega$ ) or the intercept (no variable). The symbol (+) indicates positive relationship and (-) negative relationship with the variables abundance and richness.

Models rank	Model	K	AICc	$\Delta$	$\omega$	Cumulative $\omega$
<i>(i) Richness</i>						
1	S <sup>-</sup> + SPN <sup>-</sup> + NF <sup>+</sup> + TI <sup>-</sup>	7	246.7	0.000	0.098	0.098
2	S <sup>-</sup> + NF <sup>+</sup> + TI <sup>-</sup>	6	246.9	0.279	0.085	0.183
3	S <sup>-</sup> + SPN <sup>-</sup> + NF <sup>+</sup> + NN <sup>-</sup>	7	247.2	0.501	0.076	0.259
4	S <sup>-</sup> + NF <sup>+</sup> + NN <sup>-</sup>	6	247.2	0.524	0.075	0.334
5	S <sup>-</sup> + SPN <sup>-</sup> + NF <sup>+</sup>	6	247.2	0.528	0.075	0.409
6	S <sup>-</sup> + SPN <sup>-</sup>	5	247.5	0.898	0.063	0.472
7	S <sup>-</sup> + NF <sup>+</sup>	5	247.7	1.008	0.059	0.531
8	S <sup>-</sup> + SPN <sup>-</sup> + NN <sup>-</sup>	6	247.8	1.129	0.056	0.587
9	S <sup>-</sup> + SPN <sup>-</sup> + TI <sup>-</sup>	6	247.9	1.291	0.051	0.638
10	S <sup>-</sup> + SPN <sup>-</sup> + NF <sup>+</sup> + TI <sup>-</sup> + NN <sup>-</sup>	8	248.2	1.553	0.045	0.683
11	S <sup>-</sup> + NF <sup>+</sup> + TI <sup>-</sup> + NN <sup>-</sup>	7	248.2	1.570	0.045	0.728
12	S <sup>-</sup> + NN <sup>-</sup>	5	248.6	1.956	0.037	0.765
13	S <sup>-</sup>	4	248.8	2.132	0.034	0.799
14	S <sup>-</sup> + TI <sup>-</sup>	5	249.1	2.455	0.029	0.828
15	S <sup>-</sup> + SPN <sup>-</sup> + TI <sup>-</sup> + NN <sup>-</sup>	7	249.4	2.740	0.025	0.853
16	NF <sup>+</sup> + TI <sup>-</sup>	5	250.0	3.331	0.019	0.872
17	SPN <sup>-</sup> + NF <sup>+</sup> + TI <sup>-</sup>	6	250.0	3.383	0.018	0.890
18	S <sup>-</sup> + TI <sup>-</sup> + NN <sup>-</sup>	6	250.3	3.611	0.016	0.906
19	SPN <sup>-</sup> + NF <sup>+</sup>	5	250.9	4.236	0.012	0.918
20	NF <sup>+</sup> + NN <sup>-</sup>	5	250.9	4.243	0.012	0.930
21	NF <sup>+</sup>	4	251.0	4.341	0.011	0.941
22	SPN <sup>-</sup> + NF <sup>+</sup> + NN <sup>-</sup>	6	251.2	4.507	0.010	0.951
<i>(ii) Abundance</i>						
1	S <sup>-</sup> + SPN <sup>-</sup> + NF <sup>+</sup> + NN <sup>-</sup>	7	767.4	0.000	0.574	0.574
2	S <sup>-</sup> + SPN <sup>-</sup> + NF <sup>+</sup> + TI <sup>-</sup> + NN <sup>-</sup>	8	768.7	1.292	0.301	0.875
3	S <sup>-</sup> + NF <sup>+</sup> + NN <sup>-</sup>	6	771.3	3.901	0.082	0.957

## CAPÍTULO 3

### **Impact of ivermectin use on ecological functions: the dung beetle activity**

## Abstract

The use of endectocides such as ivermectin in livestock, involves a worldwide concern due to its potential risks on non-target invertebrate fauna. Mammals cannot metabolize completely endectocides, excreting them in their faeces. Therefore, non-target insects that manipulate contaminated faeces could be affected. Our aim was to evaluate the effects of ivermectin used in the bovine livestock, on ecosystem functions performed by the native dung beetle community in Cerrado pastures. We sampled the dung beetle community in native and introduced pastures, and evaluated the effects of ivermectin use on faeces removal and soil bioturbation provided by the beetles. We collected 1572 dung beetles of 29 species. Dung beetle's richness and abundance were lower in ivermectin-treated than on ivermectin-free cattle faeces. The ivermectin use does not affect in a direct way the ecological functions (faeces removed and excavated soil). However, ivermectin use broke down the positive correlation of faeces removal with dung beetle abundance and species richness. Therefore, ivermectin-treated cattle faeces was less attractive to dung beetles and reduced dung beetle activity. We suggest that ivermectin can cause beetle intoxication, working as a partial "ecological trap".

Keywords: Cerrado, endoectocide, non-target fauna, bovine livestock, Scarabaeinae.

Highlights: Ivermectin decreases faeces attractiveness and reduces faeces removal by dung beetles, working as a partial ecological trap to dung beetles and reducing ecological functions.

## 1. Introduction

The indiscriminate use of parasiticides in bovine livestock is a worldwide concern (Bianchin et al., 1998; Kryger et al., 2005; Iwasa et al., 2007; Lumaret et al., 2007; Suárez et al., 2009). Endectocides are a family of broad-spectrum parasiticides commonly used in the control and treatment of endoparasites (e.g. helminths) and ectoparasites (eg. ticks) in livestock. One of the most used groups is the macrocyclic lactones, the group of ivermectin, doramectin and moxidectin that usually are injected in the cattle (Lumaret and Errouissi, 2002). Mammals can not metabolize completely most endectocides that are excreted within faeces, controlling the pest flies that breed in the dung (Suárez, 2009). The problem of such management arises when the excreted endectocide also affects non-target invertebrate fauna that live in soil and use the cattle faeces as food and nesting resource (Lumaret and Errouissi, 2002). The endectocide impact on non-target fauna is not completely understood and their use can be harmful, causing biodiversity loss (Lumaret and Errouissi, 2002; Römbke et al., 2010). For livestock farmers this problem can cause further losses due to ecological functions declining performed by non-target fauna such as nutrient cycling, parasite suppression and secondary seed dispersal (Wall and Strong, 1987; Wardhaugh et al., 1998, Nichols et al., 2008).

The information about biodiversity and ecological functions are essential to predict ecosystem impacts and economical human activities dependents on the environment (Armsworth et al., 2007). A considerable amount of these ecosystem functions can be quantified in terms of parasiticide use savings. Losey and Vaughan (2006) showed that dung beetles activities should be emphasized due to their role as mediators of several ecosystem functions. Dung beetles are closed linked with mammal's faeces due to their nesting behaviour (they bury their eggs with dung) and alimentary needs (Hanski and Cambefort, 1991). As a result of this faeces manipulation, dung beetles are responsible for several ecological functions: nutrient cycling, soil bioturbation and fertility and parasites suppression (Nichols et al., 2008). The ecological services, that can be defined when ecological functions are directly relevant to humans and provided economically beneficial ecosystem services (Nichols et al., 2008) provided by dung beetles were estimated at 380 million dollars per year in USA (Losey and Vaughan, 2006). By burying cattle faeces, dung beetles remove dung from pastures, reducing forage fouling and limiting livestock helminths parasitism and haematophagous flies attacks (Losey and Vaughan, 2006).

Brazil has one of the largest bovine livestock in the world (FAO, 2008) and the majority of the pastures are placed in the Cerrado region (Martha Junior and Vilela, 2002; Silva et al., 2006). The Cerrado is the second largest biome of Brazil (Alho, 2005) and the largest woodland-savannah of the American continent (Furley, 1999). Furthermore, it is one of the most species rich savannas in the world with high levels of endemism (Myers et al., 2000; Klink and Machado, 2005). However, only 2.2% of Cerrado area is being protected (Klink and Machado, 2005). The conversion of native grasslands to monocultures for commodities production (Queiroz, 2009) and pastures (Klink and Machado, 2005) is among the major threats against Cerrado biodiversity (Bond and Parr, 2010).

The Brazilian government has been providing incentives to modernize of agricultural lands during the last decades (Silva, 2000) due to the presumed higher carrying capacity of exotic grass pastures (Martha Junior and Vilela, 2002). The modernization includes the replacement of native grasslands for introduced exotic grass pastures (personal contact, EMATER- Company of technical support and rural extension-Carrancas), and as alternative to negate fire management, considered a traditional way to manage native pastures (Pivello and Coutinho, 1996). The most common exotic grass used to replace the native grassland is the African grass *Urochloa* spp. (*Urochloa* P. Beauv. spp. (= *Brachiaria* (Trin.) Griseb. spp.) due to its tolerance to low fertility and acid soils, characteristics of Cerrado (Martha Junior and Vilela, 2002).

The replacement of native to exotic pastures on dung beetle community was studied by Almeida et al. (in press) and this organisms have been widely used in ecological researches that assess both land-cover changes (e.g. Shahabuddin et al., 2005; Gardner et al., 2008, Louzada et al., 2010) and endectocides on non-target fauna (e.g. Lumaret and Errouissi, 2002, Suárez, 2002; Römbke et al., 2010). However, the studies made in Brazil only tested the effect of endectocides on the exotic African dung beetle *Digitonthophagus gazella* in pastures of introduced grass species or through bioassays due to its economical value (Bianchin et al., 1992, 1998; Pratisoli and Torres, 1998).

This paper focuses on the effects of ivermectin used in the bovine livestock on ecological functions performed by native dung beetle community. We tested the hypotheses that the use of ivermectin reduces the amount of cattle faeces removed and excavated soil, that promote nutrient cycling and soil bioturbation thought the lower faeces attractiveness for dung beetles.

## 2. Materials and methods

### 2.1. Study sites

Our study sites were located in the municipality of Carrancas, south of Minas Gerais State, southeastern Brazil (21°28'24" S, 44°39'05"W). The region is considered a transition between Cerrado and Atlantic forest biomes (Oliveira Filho et al., 2004) due to the landscape constituted by a matrix of Cerrado grasslands and forests, and riparian semi-deciduous forests, that present elements of the Atlantic forest biome. The altitude varies between 970m and 1050m. The climate is Cwa according to Koppen classification, and the region has a mean of 1480mm annual precipitation and a mean annual temperature of 15° C (Oliveira-Fillho et al., 2004).

The south of Minas Gerais is one of the most important milk producing region in Brazil (Zoccal et al., 2006) and the majority of producers are smallholders that have selling milk production to dairy factories as their main economical activity (IBGE, 2006). Carrancas municipality groups all those characteristics (IBGE, 2006) and it is also part of the traditional “Fine Cheese Circuit” in Brazil (Leandro, 2008), often considered as resulting from higher-quality milk from cattle raised on more nutritious native grassland pastures.

The smallholders used to utilize *campo limpo* (native Cerrado grasslands) as extensive pastures (Fontanelie and Jacques, 1988; Jacques, 2003). Native grasslands are composed of several native herbaceous species, with predominance of grasses (Poaceae) and Leguminosae (Carvalho, 1993; Rodrigues and Carvalho, 2001; Munhoz and Felfili, 2006).

We sampled eight native (mixed native plants) and eight introduced pastures (monoculture of *Urochloa* spp.), the most common pastures used in the region, at least 300m apart from each other. We carried out our study during February 2010, which corresponds to the rainy season, when dung beetles present maximum abundance in the tropics (Martínez and Vásquez, 1995; Milhomem et al., 2003).

### 2.2. Ivermectin treatment

Girolando cattle, a mix of *Bos indicus* (Gir cattle) and *Bos taurus* (Holstein cattle) are the most often used species in dairy farms in southeast Brazil (Barbosa, 2006). We selected six girolando cows at a dairy farm in Carrancas and did

subcutaneous injections of 1% ivermectin solution, so as to attain 0.2 mg/kg of live weight (Suarez, 2002). Ivermectin is a parasiticide widely used in Brazil and other countries (Kryger et al., 2005; Bianchin et al., 1998; Lumaret et al., 2007; Suárez, 2009). After five days, the peak period of ivermectin action (Suarez, 2002; Lumaret et al., 2007, Römcke et al., 2010), we collected fresh cow dung each day to bait our traps and measure the ecological functions provided by dung beetles. The cows were kept in a separated pasture to maintain their faeces contaminated with ivermectin apart of other faeces free of the endectocide. We also collected fresh cow dung from cows not injected with ivermectin for our control treatment.

### *2.3. Evaluation of ecological functions*

We collected data on ecological functions in each of the sampled pasture. We delimited a 1m-diameter circumference with a 20cm high net placed at soil level with small sticks to limit the movements of dung beetles. At the centre of this “arena”, we put 500g of fresh cow faeces. Six arenas separated by 100 m in a rectangle design were placed on each pasture: three arenas contained cow faeces with ivermectin and three arenas free of ivermectin. After 24h we measured the ecological functions that consisted in the nutrient cycling (amount of cow faeces removed) and the bioturbation (excavated soil) performed by dung beetles. We assumed that some cow faeces had been removed by dung beetles because of the small rounded holes present near the faeces and of their visual presence. Dung beetles can be classified in functional guilds according to their faeces manipulation. The (1) rollers handle dung by rolling it away to bury it far from the faeces pad; (2) tunnelers handle dung by tunneling vertically underneath the dung and burying a portion of it below ground; (3) dwellers simply lay their eggs directly into the dung pad (Hanski and Cambefort, 1991). The net limiting the arena helped the contention of the faeces dispersed by dung beetle. The rollers dung beetles were forced to dig the soil to bury the dung ball or to abandon the ball in the arena limits. The tunnellers bury the dung ball underneath the pad of cow faeces or nearby the faeces pad. We collected and measured the total amount of faeces removed from the main pad and the cow dung pad remnants and weighed them in the lab. The soil excavated by dung beetles in the limits of the arenas was collected in bags and also weighed in the lab. This “arena technique” has been developed and used with success in the work of Braga (2009) with the dung beetles of Amazon to measure the ecological functions provided by dung beetle.

In order to have a control of humidity loss of faeces, a small sample of cow faeces (20g) was placed in a small container (diameter 4 cm, height 4 cm) covered with a net to avoid flies attacks which would overweight our humidity control. The container was bound to a stick placed near each arena to measure humidity. After 24h, we weighed the remaining faeces of humidity control. We considered the final weight of humidity control as the percentage of water lost. In our measures, we considered this percentage of humidity loss to estimate the final weight of cow faeces left in the centre of the arenas. In total, there were 96 arenas in our study.

#### *2.4. Dung beetle sampling*

After the 24h-period during which we measured the ecological functions, we sampled the dung beetles in the same sites as where we placed the arenas to evaluate the dung beetle community responsible for cow dung removal soil excavation. Dung beetles were sampled using pitfall traps (19cm diameter, 11cm depth) buried flush with the ground and filled with 150ml of saline solution and detergent. The trap has an iron stick fixed in the soil in which we attached a bag made with a net to accommodate the bait constituted by 500g of fresh cow faeces. Six traps were placed in each pasture, separated by 100 m in a rectangle design (see Almeida et al., submitted for a similar design). We placed, alternately, three traps with cow faeces contaminated with ivermectin and three traps with cow faeces free of ivermectin. We had 96 traps in the study. Trapping was conducted for 24h in each pasture.

Dung beetles were identified to genus and species when possible, using the reference collection of Invertebrate Ecology and Conservation Laboratory (IEC) at Universidade Federal de Lavras (UFLA), Brazil. Voucher specimens were placed at the IEC and at Universidade Federal do Mato Grosso (UFMT- Brazil) collection.

#### *2.5. Statistical analyses*

We used generalized linear mixed-effects models (GLMMs) and, “pasture” as random effect to examine the effects of ivermectin treatment and pasture systems on dung beetle richness and abundance. In order to detect the effects of richness and abundance on ecosystem functions provided by dung beetles (faeces removal and excavated soil), we also used generalized linear mixed-effects models (GLMMs)

and, “pasture” as random effect. Complete models were adjusted by excluding non significant variables (Crawley, 2007) and Poisson distribution, corrected for overdispersion (Crawley, 2007, Zuur et al. 2009). All these analyses were undertaken within the R environment (R Development Core Team, 2009).

We used non-metric multidimensional scaling (NMDS), using the Bray-Curtis index on log-transformed abundance matrix to explore differences in community species composition across the pasture systems and the treatments with ivermectin. The stress value is used to assess the robustness of the NMDS solution; stress values above 0.2 indicating plots that could be unreliable (Clarke, 1993). Analysis of similarity (ANOSIM; Clarke, 1993) was used to test for significant differences in multivariate community structure. ANOSIM is a non-parametric permutation test for similarity matrices that is analogous to an ANOVA. These analyses were conducted in Primer v. 5 (Clarke and Warwick, 2001).

### **3. Results**

We collected a total of 1572 dung beetles belonging to 29 dung beetles species, distributed across six tribes and 14 genera. As for functional guilds, the majority of collected dung beetles were tunnellers (Table 1). In native grassland pastures we collected 736 individuals of 28 dung beetles species; in introduced pastures we collected 836 individuals of 21 dung beetles species. Traps containing ivermectin-treated cattle faeces collected 555 individuals of 20 species; in ivermectin-free cattle faeces collected 1017 individuals of 24 species. An amount of 236.28g (mean/per pasture) of cow faeces was removed in control treatment and 215.08g (mean/per pasture) of faeces in ivermectin treatment. The amount of soil excavated related with control treatment was 619.18g (mean/per pasture) and 433.89g (mean/per pasture) in ivermectin treatment.

#### *3.1. Dung beetle attractiveness*

Dung beetle's abundance ( $\chi^2= 137.8$ ,  $P<0.001$ ; Fig.1) and species richness ( $\chi^2=4.97$ ,  $P=0.03$ ) were lower in ivermectin-treated faeces, Fig. 1). Pasture system did not affect dung beetle abundance ( $\chi^2<0.001$ ,  $P=0.94$ ), nor species richness ( $\chi^2=0.67$ ,  $P=0.41$ ).

### 3.2. Species composition

Dung beetle species composition was not altered by ivermectin use (NMDS stress value=0.33, ANOSIM, R=0.05, P>0.05) nor pasture system (NMDS stress value=0.33, ANOSIM: R=0.06, P>0.05). Ivermectin use did also not affect species structure within native pastures alone (NMDS stress value=0.27, ANOSIM: R=0.10, P>0.05), nor within introduced pastures alone (NMDS stress value= 0.23, ANOSIM, R=0.01, P>0.05).

The most abundant species were *Dichotomius bos* and *Dichotomius nisus* in both pasture systems and parasiticide treatment (Table 1). Only *Ateuchus puncticollis* presented more individuals in cow faeces with ivermectin.

### 3.3. Ecological functions

Cow faeces removal increased with number of individuals and had an interaction with ivermectin treatment that remains around 280g of faeces removed with the treatment ( $\chi^2=9.80$ , P=0.001, Fig.2A). Also, the faeces removed increased with number of species and had statistical interaction with ivermectin treatment that remains stable in an amount of 200g of faeces removed ( $\chi^2=5.32$ , P<0.05, Fig.2B). The quantity of faeces removed ( $\chi^2=21.66$ , P<0.001) was correlated with amount of excavated soil (Fig.3). The number of individuals ( $\chi^2=8.33$ , P<0.01, Fig.4A) and number of species ( $\chi^2=6.45$ , P<0.05, Fig.4B) had positive influence upon the amount of excavated soil.

## 4. Discussion

### 4.1. Abundance and richness

Replacement of native grassland pastures by introduced exotic grass pastures did not influence the number of individuals and species, probably because the replacement does not influence resource availability for this community.

Overall, we collected the same number of species than other studies in Cerrado's pastureland using cow faeces as bait (Oliveira et al., 1996; Koller et al., 1999; Marchiori et al., 2003; Louzada and Silva, 2009), but contrastingly less than in another dung beetle study in the same site. Almeida et al. (in press) found three times more individuals and twice the number of species in the same pasturelands. This contrast, probably, is due to

the higher effort (more than two times the number of traps) and the use of human faeces as bait. Previous studies showed that cow faeces attract only a limited suite of dung beetle species, which are able to use this resource: cow faeces (Dormont et al., 2004; Louzada and Silva, 2009).

We accepted our hypothesis that ivermectin use on cattle has a negative effect on dung beetle's richness and abundance. Faeces of ivermectin-treated cows presented lower individual and species numbers. This indicates that the ivermectin that remains in the faeces reduces its attractiveness to dung beetles. Previous studies, reviewed by Suárez (2002), found contradictory results. Dung pads with ivermectin can be less attractive, have no effect, or even be more attractive to dung beetles species.

Römbke et al. (2010) suggested that attractiveness depends on dung beetle species and ivermectin concentration. As far as the majority of studies on the subject use the same ivermectin concentration, recommended by the endectocide producer in a 1% ivermectin concentration (0.2 mg/kg), we discard this explanation for our study. One of the 29 dung beetle species, *Ateuchus puncticollis*, responded positively to ivermectin treatment. Maybe the South-african dung beetle fauna, that did not respond to ivermectin (Kryger et al., 2005), and the European fauna that was attracted to ivermectin (Römbke et al., 2010), comprehended species that present autecological differences to the Cerrado dung beetle fauna.

Based on our results, and their striking divergence to those of Kryger et al. (2005) and Römbke et al. (2010), we suggest that the negative effects of ivermectin on dung beetle abundance and species richness are characteristic of the Cerrado's dung beetle fauna, and therefore, results from other biogeographical regions cannot be generalized.

#### 4.2. *Species composition*

Of the 29 dung beetle species collected in this study, 25 were also present in human faeces used as bait, in the same sites (Almeida et al., submitted). This fact could be evidence that dung beetles able to explore cow faeces are a subset of dung beetle community that usually use native mammal's faeces as food resource and be considered generalists.

We did not detect any effect of pasture introduction on dung beetle species composition. Ivermectin use did not affect dung beetle species composition. Kryger et al. (2005) showed that ivermectin did not affect the community structure or composition

in a long-term field trial. Krüger and Scholtz (1998 a,b) found that ivermectin led to changes in dung insect communities just under drought conditions, but not under high-rainfall conditions. However, our study was carrying out in the middle of rainy season and the same pattern was found.

The dominance of *Dichotomius bos* corroborate similar patterns found in Brazilian pasturelands. *D. bos* is the most often dung beetle species collected in pastures, representing the predominance of tunnellers in this habitat (Marchiori, 2000; Marchiori et al., 2003; Mendes and Linhares, 2006; Koller et al., 2007; Louzada and Silva, 2009) .

#### 4.3. Ecological functions

Our work showed that faeces removal was not directly influenced by ivermectin treatment due to the stabilization of the amount of faeces removed and the number of dung beetles species and individuals that provided these ecological functions did not influence this amount. According to our results, the presence of ivermectin breaks down the correlation between faeces removal and dung beetle abundance and richness. Dadaour et al., (1999) verified such break down in studying single dung beetle species abundance, *Onthophagus taurus*.

Our results show that even ivermectine-treated faeces were removed, up to a certain amount. However, an increase in beetle numbers does not lead to increased faeces removal. We interpret this as resulting from two sequenced processes. Those beetles that were attracted to the baits initiate faeces removal, in both ivermectin-treated and ivermectin-free baits. However, the dung beetles in the ivermectin samples reduce, or stop, their activity, after some time, probably as a result of intoxication. Several studies have focused on lethal (adult and larval mortality) and sub-lethal (reproductive injuries) effects of endectocides on individual dung beetles species (Bianchin et al., 1998; Iwasa et al., 2007; Bang et al., 2007) but under field conditions we can not affirm the real effect of ivermectin on individuals and on their ecosystem functions. However, Galbiati et al. (1995) tested under lab conditions, the effect of ivermectin on *Dichotomius anaglypticus*, (Mannerheim, 1829), (= *Dichotomius bos*, Blanchard, 1843) and faeces removal and found decrease in the rate of faeces removal and death of individuals. Hence, we only can suppose that the ivermectin could promote the *D. bos* mortality or intoxication of the most abundant species in our study.

Overall, each cow can produce a mean of 32 kg of faeces per day (Costa 1989). As we showed in our study, in 24h-period, in a limited space of 1m-diameter, native dung beetles were able to remove completely the 500g of faeces left in the arenas, in patches with high number of individuals and species. The accumulation of faeces in pastures due to low faeces attractiveness on dung beetles and low dung beetle performance in ivermectin-treated faeces can generate the interruption of faeces decomposition due to non-activity of dung fauna (Wall and Strong, 1997; Suárez et al., 2003; Iglesias et al., 2005; Römbke et al., 2010). Consequently, the forage is rejected by cattle (Arnold 1981) and the bioturbation interrupted due to non-faeces removal provided by dung beetles also brings consequences as loss of soil porosity and humidity in pasturelands (Brown et al., 2010), leading to economic losses to farmers.

## **5. Conclusion**

There was no direct effect of ivermectin use on ecological functions. However, the ivermectin use broke down the positive correlation of faeces removal with dung beetle abundance and species richness. Moreover, the ivermectin-treated cattle faeces were less attractive to dung beetles. We hypothesize that stagnation of dung beetle performance was due to beetle intoxication with ivermectin, working as a partial “ecological trap”. We concluded that ivermectin reduces ecological functions through the reduction of faeces attractiveness for dung beetles. Kryger *et al.* (2005) considered the use of ivermectin safe under high rainfall conditions in Africa. Our results showed the opposite for Brazilian Cerrado: we detected interference of ivermectin on dung beetles and on their ecological functions in the rainy season, when the majority of farmers utilize ivermectin to suppress parasites as horn fly (Bianchin and Alves, 2002), helminths and ticks (Carvalho et al., 2002).

In spite of the adverse effects on dung attractiveness, dung beetles still were attracted by contaminated dung and the ecological functions were not completely suppressed. However, these beetles presented a diminished activity, which may result from intoxication or even mortality. This would imply that ivermectin-treated cow faeces works as a partial ecological trap. However, under field conditions we can not affirm the real effect of ivermectin on individuals and further studies are necessary to analyze the direct effect of ivermectin on native dung beetles.

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Table 1. Table of mean abundance per pasture (N=16), of dung beetles (separated by tribes and functional guilds) collected in native and introduced pastures and in traps containing faeces with and without ivermectin, in Carrancas, Minas Gerais, Brazil.

Tribe/ Species /Guild	Mean abundance			
	Native	Introduced	Ivermectin	Control
<b>Ateuchini (Tunnellers)</b>				
<i>Ateuchus aff. puncticolis</i>	5.12	5.25	7.25	3.12
<i>Ateuchus striatulus</i>	3.37	1.31	2.31	2.37
<i>Ateuchus subquadratus</i>	0.06	0	0.06	0
<i>Ateuchus vividus</i>	1.06	8	0.43	1.12
<i>Canthidium barbaticum</i>	0.06	0.06	0	0.12
<i>Canthidium decoratum</i>	0.06	0.25	0.06	0.25
<i>Generidium cryptops</i>	0.06	0.12	0.18	0
<i>Trichillum adjunctum</i>	2.18	4.93	2	5.12
<i>Trichillum extenepuctatum</i>	0.06	0.06	0.18	0.5
<i>Uroxys sp.1</i>	0.12	0	0	0.12
<b>Canthonini (Rollers)</b>				
<i>Agamopus viridis</i>	0.43	0.18	0.31	0.31
<i>Agamopus unguicularis</i>	0.81	0.18	0.37	0.62
<i>Canthon aff. dives</i>	0.06	0	0	0.06
<i>Canthon aff. janthinus</i>	0.06	0	0	0.06
<i>Canthon aff. unicolor</i>	0.43	0.12	0.06	0.5
<i>Deltochilum elevatum</i>	0.31	0.25	0	0.5
<i>Deltochilum sp.2</i>	0.12	0.12	0.06	0.18
<i>Deltochilum pseudoicarus</i>	0.06	0	0	0.06
<b>Coprini (Tunnellers)</b>				
<i>Dichotomius bos</i>	15.37	20.81	14.06	22.12
<i>Dichotomius crinicollis</i>	0.12	0	0	0.12
<i>Dichotomius luctuosus</i>	0.31	0.43	0.31	0.43
<i>Dichotomius nisus</i>	9.93	11.25	3.18	18
<i>Dichotomius semiaeni</i>	0.25	0.25	0.18	0.31

Tribe/ Species /Guild	Native	Introduced	Ivermectin	Control
<i>Isocoprís inhatus</i>	0.18	0.18	0.12	0.25
<i>Ontherus appendiculatus</i>	3.75	4.81	2.93	5.62
<i>Ontherus digitatus</i>	0.06	0	0	0.06
<b>Eurysternini (Dwellers)</b>				
<i>Eurysternus parallelus</i>	0.06	0	0.06	0
<b>Onthophagini (Tunnellers)</b>				
<i>Onthophagus aff. hirculus</i>	0.87	1.06	0.43	1.5
<b>Phanaeini (Tunnellers)</b>				
<i>Phanaeus kirby</i>	0	0.06	0	0.06

Figure 1. Observed mean richness and abundance of dung beetles (per pasture, N=16) between ivermectin treatment and control (\* means  $P < 0.05$  and \*\*\*  $P < 0.001$ ) based on generalized linear mixed model (GLMM) using “pasture” as random effect.

Figure 2. Relationship between faeces removed (g), abundance of dung beetle (log-transformed), number of species with treatment with ivermectin in Cerrado’s pastures based on generalized linear mixed model (GLMM) using “pasture” (N=16) as random effect.

Figure 3. Correlation of faeces removed (g) and amount of excavated soil (g) by dung beetles in Cerrado’s pastures based on generalized linear mixed model (GLMM) using “pasture” (N=16) as random effect.

Figure 4. Relationship between excavated soil by dung beetles (g) between number of species and of dung beetle abundance (log-transformed) in Cerrado’s pastures based on generalized linear mixed model (GLMM) using “pasture” (N=16) as random effect.

Fig.1

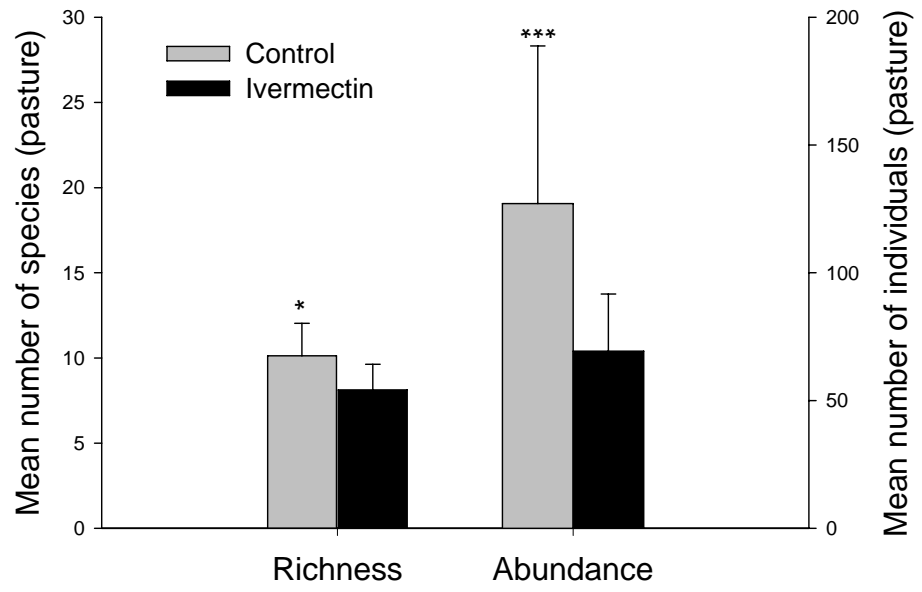
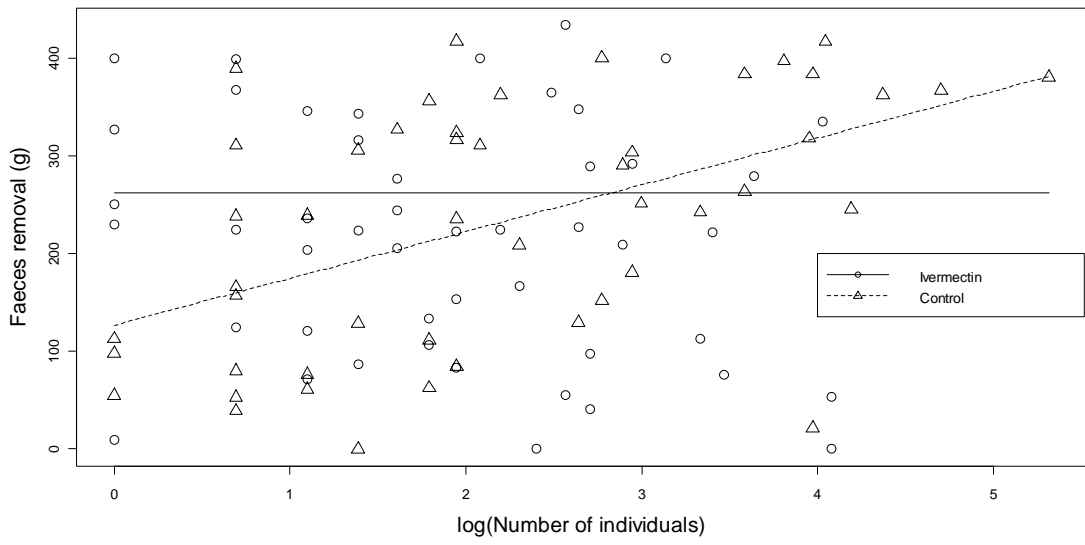
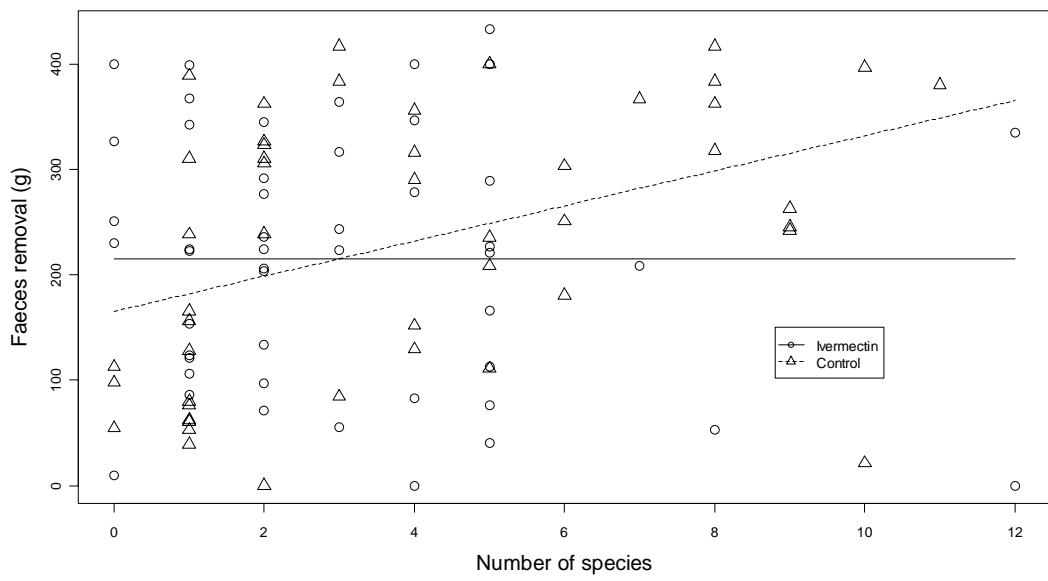


Fig.2



**A**



**B**

Fig. 3

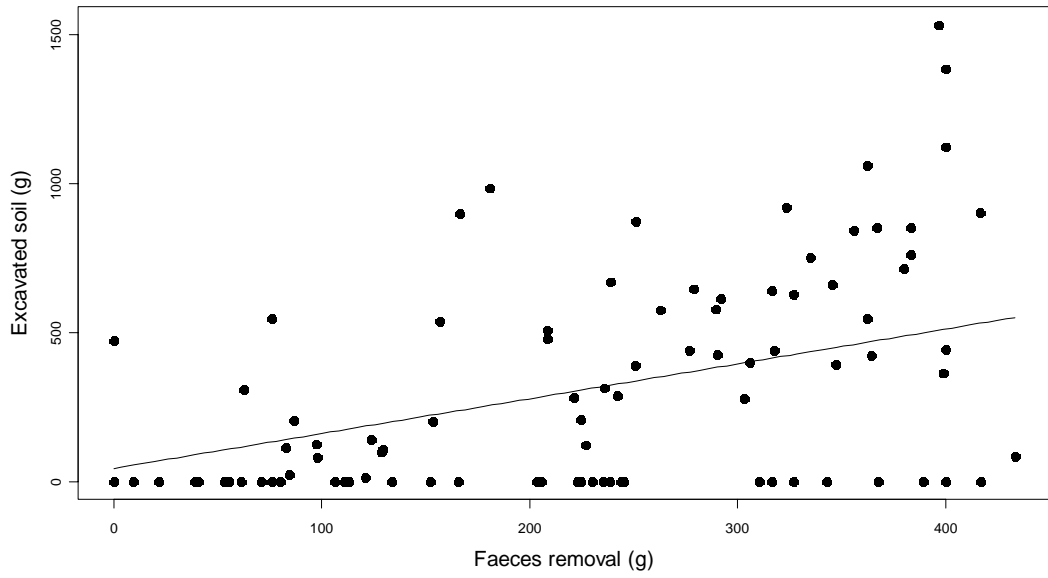
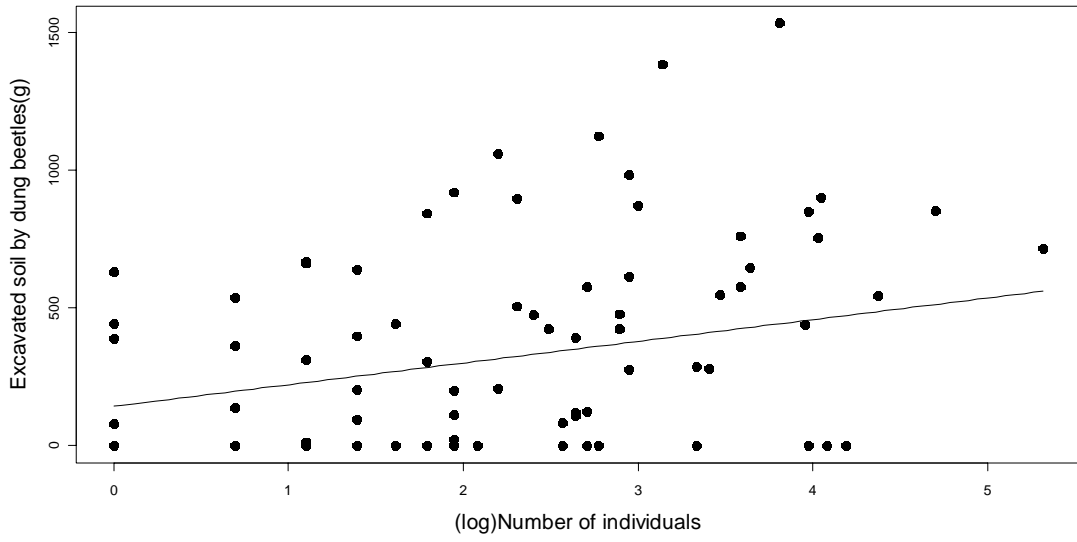
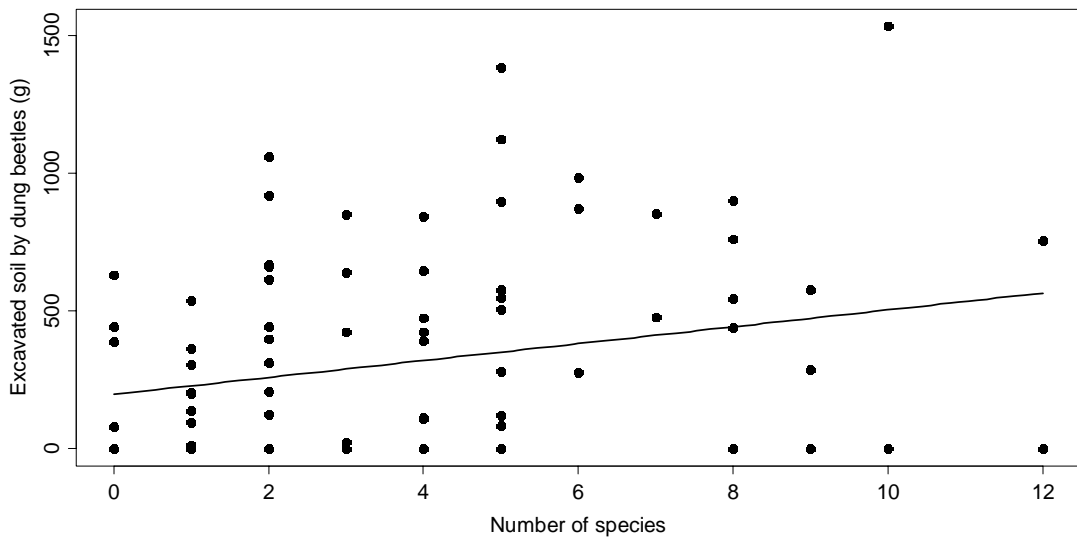


Fig.4



**A**



**B**

## CONCLUSÃO GERAL

Nós concluímos que a substituição das pastagens nativas em pastagens de braquiária acarreta em perda de diversidade biológica, mudanças em características do solo e a uma estagnação das funções ecológicas desempenhadas pelos escarabeíneos devido ao uso de ivermectina e, portanto, acarretando em prejuízo para a paisagem agropastoril do Cerrado.

No contexto de Carrancas, uma área de Cerrado, a maior parte da sua vegetação nativa (Scolforo *et al.* 2008), e cerca de 90% das fazendas ainda possuem pastos nativo (IBGE 2006). Essa região onde a cidade se situa é considerada uma das áreas prioritárias para conservação em Minas Gerais devido a sua grande biodiversidade animal e vegetal (Drummond *et al.* 2005). Entretanto, como em outras regiões do Cerrado, a ameaça de degradação de áreas naturais estão aumentando. Órgãos do governo, como a EMATER ainda incentivam os produtores a implantar braquiária em suas fazendas com o intuito de aumentar a capacidade de suporte dos pastos (Martha Júnior & Vilela 2002) e também para evitar o manejo com o fogo nos campos nativos. Como vimos em nosso trabalho, o fogo afeta muito pouco a comunidade de escarabeíneos se comparado com o processo drástico necessário à implantação de braquiária. Além disso, o manejo feito com fogo é regularizado por lei e uma série de procedimentos de segurança devem ser levados em consideração ao utilizar o fogo nas pastagens nativas (Minas Gerais 2004). O problema que ocorre é que frequentemente os produtores levam meses para conseguir a permissão para fazer o manejo com o fogo do IEF-MG (S. Almeida, pers.obs.). Esse atraso para a obtenção da licença no período correto do ano (começo e final da estação chuvosa) acaba por levar os fazendeiros a optar por manejar suas terras, fazendo assim, a introdução da braquiária que não necessita de fogo para ser manejada (observação pessoal).

Mudanças na lei que não forneçam mais subsídios para a conversão das pastagens nativas e tornem o manejo com o fogo bem fiscalizado e menos burocrático podem ajudar a prevenir a perda da diversidade biológica nos campos naturais de Cerrado. A experiência obtida com esse trabalho também sugere que uma comunicação esclarecedora com os produtores pode ajudar em mudança nas práticas de manejo. No caso de Carrancas, a interação que ocorreu com os pequenos produtores de leite mostra que não existe informação a respeito da importância dos besouros escarabeíneos para as pastagens como a ciclagem de nutriente promovida pela remoção das fezes nos pastos, a bioturbação que promove aeração e umidade do solo, assim como no controle de

parasitas, como as moscas e os helmintos (Nichols *et al.* 2008). A disseminação destas informações sobre os benefícios dos pastos nativos e da sua biodiversidade (tendo como modelo os escarabeíneos) pode contribuir para uma mudança no entendimento dos produtores a respeito do meio em que vivem, ajudando assim, na sua preservação. A maioria dos estudos ecológicos têm sido realizados em áreas protegidas (Hilty & Merenlender 2003) e as áreas de Cerrado não são uma exceção (Brannstrom 2003), como evidenciado pela falta de informação a respeito da biodiversidade dos insetos no Cerrado, tanto em campos nativos quanto em campos cultivados. Estudos em áreas privadas são essenciais para o entendimento das reais ameaças para a diversidade regional (Estrada & Coates-Estrada 2002, Hilty & Merenlender 2003) nesse complexo mosaico de habitats que é o Cerrado (Ratter *et al.* 1997).

O compartilhamento de informação científica com os produtores pode ser uma boa fonte de interação. A colaboração de pesquisadores, órgãos governamentais e fazendeiros deve ser encorajada através das políticas de uso da terra no Cerrado (Brannstrom *et al.* 2008) que pode contribuir na manutenção das pastagens nativas nas fazendas, e conseqüentemente, na manutenção da diversidade regional. Um exemplo pouco conhecido é o ICMS ecológico. Uma parte do dinheiro provindo do ICMS estadual é repassado para cidades que possuem maior número de áreas conservadas (Minas Gerais 2000). Um esforço por parte das prefeituras para promover essa lei entre os fazendeiros, gerando uma alternativa para a conservação da diversidade regional e um incentivo ao desenvolvimento econômico.

Dessa forma, os resultados desse trabalho ressaltam a importância da manutenção das pastagens nativas para a paisagem agro-pastoril do Cerrado e revela que a introdução de pastagens de braquiária é causadora de uma reestruturação da comunidade de escarabeíneos, que acarreta numa estagnação de importantes funções ecológicas, tanto devido à diminuição da riqueza e abundância de indivíduos pela introdução, assim como pelo uso da ivermectina no manejo do gado leiteiro.

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