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#### Research Article

# The influence of feral horses dung piles on surrounding vegetation

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#### Abstract

The effect of grazing by large herbivores on grassland communities has been extensively studied, however less is known about changes associated with sites of high-intensity activity, such as dust bowls, tracks, urine patches and dung piles, that might induce disproportionate impacts to the directly affected areas and the surrounding vegetation. This paper explores the changes associated with exotic feral horse dung piles in relicts of natural grasslands in Argentine Pampas. We expected greater changes in the composition of plant communities and a greater facilitation effect on the establishment of invasive alien plants adjacent to the dung piles. Characteristics of the vegetation surrounding dung piles were recorded in 100×25 cm plots located 0–1.5 m from the edge of the piles. We compared the immediate surroundings with reference plots at 5 m. The diversity of plant species increased as distance from the edge increased from 0–1.5 m; however, species richness was significantly higher in the first meter next to dung piles than 5 m away. Percentage cover of bare ground decreased further from the dung piles at both scales. Percentage cover of woody plants was greater at greater distances from the manure, whereas percentage cover of exotic plants was significantly higher next to the dung piles. The reported changes could be related to concentration gradients of nutrients liberated from the manure and/or to behaviour patterns of the horses, which may avoid grazing in the immediate surroundings of dung piles. These changes result in invasion windows facilitating the establishment and subsequent dispersal of exotic plant species in grasslands.

Key words: invasive alien species, biodiversity, invasion window, exotic herbivores, natural grasslands

## Introduction

The occurrence of disturbances caused by animals on a small scale could be an important process in determining the floristic diversity of a region as they provide temporary windows for the establishment of uncommon species (Branch et al. 1999; Fields et al. 1999; Farji-Brener and Ghermandi 2000). The effect of large herbivores, whether domestic, feral or wild, has been mainly focused on the consequences of grazing and trampling on the composition and diversity of plant communities (Vázquez 2002; Rook et al. 2004), but very little is known about the impact of intense disturbances at smaller scales, such as dust bowls, tracks, urine and dung patches, on the vegetation (Day and Detling 1990; Steinauer and Collins 1995; Loydi and Zalba 2009).

Dust bowls and tracks open up areas of bare ground, which favours species adapted to frequent disturbances by providing microsites that are free of vegetation (Grime 2001; Bakker and Olff 2003; Renne and Tracy 2007), and increases the risk of erosion at the same time (Cole and Spildie 1998). Latrine areas are intensely supplied with urine resulting in a local increase in nutrients, which gives rise to variability in productivity and species diversity (Semmartin and Oesterheld 2001; Zhi-You et al. 2004; Renne et al. 2006). Herbivores, on the other hand, help to disperse whole groups of species frequently found in association with dung piles (Campbell and Gibson 2001; Cosyns and Hoffmann 2005; Wells and Lauenroth 2007). The dung piles also provide sites appropriate for seedling establishment (Malo and Suárez 1995; Dai 2000), increasing recruitment

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of plant species exotic to the biome growing directly on the dung (Loydi and Zalba 2009).

These disturbances are expected to be particularly intense in habitats that have not shared an evolutionary history with the herbivores responsible for these selective pressures (Mack 1989). The presence of large introduced ungulates might favour the establishment of opportunist exotic plant species in these "naive" ecosystems (Grime 2001).

In a previous study in this area, species richness and percentage cover of exotic species growing directly on dung piles were shown to be significantly higher than in control areas with natural vegetation (Loydi and Zalba 2009). Considering this, dung piles were proposed as "nursery areas", allowing the introduction and initial survival of invasive species that could eventually spread into more pristine habitats. The effect of dung piles is not restricted to sites directly affected by the dung but extends to the surroundings, creating environmental gradients that result in changes in the dynamics of species recruitment and survival, eventually leading to changes in the floristic diversity of a whole area (Olff and Ritchie 1998). These changes may include the spread of unwanted species including non-palatable and invasive alien plants. In this paper we propose that dung piles of feral horses result in changes in the composition of the surrounding plant communities, including the facilitation of the establishment of invasive alien plants. To test this hypothesis we described the composition and diversity of plant species communities at different distances from feral horse dung piles, and compared the abundance of exotic species in the immediate vicinity of the piles with plots located away from them.

#### Methods

Study area

The study was carried out at Ernesto Tornquist Provincial Park (ETPP), in Ventania mountains, Argentina, which covers an area of approximately 6700 ha, including elevations of over 1000 m, between 38°00' and 38°10'S, and 61°45' and 62° 08'W. Climate is temperate, with a mean annual temperature of 14°C. Mean annual precipitation is 800 mm, with occasional snow in winter (Burgos 1968). The main vegetation is grassland, dominated by *Stipa*, *Piptochaetium*, *Festuca*, and *Briza* (Cuevas and Zalba 2010). The shrubs *Discaria americana* and *Eupatorium buniifolium* 

are the dominant woody species (de Villalobos et al. 2011). Native trees are absent in the system (Zalba and Villamil 2002). The study area consists of valleys with slopes of 5 to 11 %, shallow soils and occasional rocky outcrops. In the absence of intensive grazing, habitats in the foothills are characterised by grassland of 50–60 cm in height (de Villalobos and Zalba 2010).

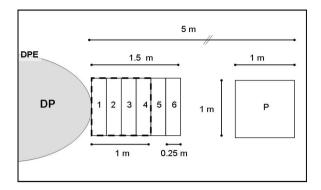
ETPP is one of the last relicts of pampas grassland in Argentina and possibly the most valuable for its concentration of endemic species and of populations of plants and animals that are seriously threatened in the rest of the region (Kristensen and Frangi 1992; Bilenca and Miñarro 2004).

In 1942 a small group of horses was introduced into the park, growing until being the largest known feral horse population in Argentina. Adult density doubled from 1995 (10.9 animals km<sup>-2</sup>) to 2002 (21.0 animals km<sup>-2</sup>, Scorolli and Lopez Cazorla 2010). At the time of this work, total horse density in the park was ca. 23 animals km<sup>-2</sup> (A.L. Scorolli, Universidad Nacional del Sur, pers. com.). Their impact on the vegetation is notable, reducing vegetation biomass, replacing species (de Villalobos and Zalba 2010; Loydi et al. 2012) and promoting the advance of invasive plants (Loydi et al. 2010; de Villalobos et al. 2011), and their presence is also associated with changes in avian communities and reductions in the reproductive success of grassland birds (Zalba and Cozzani 2004).

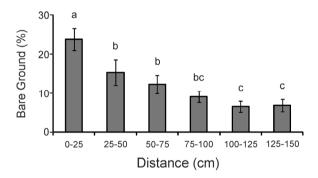
#### Experimental design and statistical analysis

Sampling was carried during the austral summer (January and February), in an 86.31 ha valley used by feral horses. In a previous study the percentage of ground covered by horse manure in this area was estimated at 2.5% (Loydi and Zalba 2009).

Fifteen dung piles of more than one metre in diameter and separated by at least 100 m were selected and four sets of six adjacent 0.25 m² plots (100cm × 25cm) each were placed from 0–1.5 m from the dung pile edge (Figure 1). These were oriented according to the major and minor lengths of the dung piles assuming an oval shape (see Loydi and Zalba 2009 for details). Where the terrain made it difficult to place the plots, the number of sets of plots was reduced from four to three. Four 1 m² plots were also placed five metres away from the dung pile edge, in line with the smaller plots. The vegetation of these plots was compared to that of the four contiguous



**Figure 1.** Plan of the plots associated with each dung pile. The discontinuous line shows a combination of the four 4  $0.25 \text{ m}^2$  plots nearest to the dung pile. 1 to 6: plots of  $0.25 \text{ m}^2$ ; DP: Dung Pile; DPE: Dung Pile Edge; P: plot of 1 m<sup>2</sup> at 5 metres from the DPE.



**Figure 2.** Mean percentage of bare ground in plots in the smaller gradient. Vertical bars represent standard error. Different letters indicate significant differences between the means (Tukey test p < 0.05).

plots nearest the dung piles pooled together. In this way we analyzed a small gradient of six plots covering 1.5 m next to each dung pile and a larger gradient for comparing the vegetation next to the dung piles with that at a distance of 5 m.

All vascular plant species present in the plots, including native and exotic species, were recorded and percentage cover was visually estimated for each one, as well as percentage of bare ground. Species richness and Shannon diversity index were calculated with these data (Krebs 1998). Changes in species richness, Shannon diversity index and percentage of bare ground in plots at different distances from the dung pile edges were evaluated using a one-way analysis of variance (ANOVA) with a block design (Steel and Torrie

1985; Zar 1996). Each dung pile was considered a block as it was already known that the associated vegetation would vary with the time since abandonment of the pile by horses (Loydi and Zalba 2009). All sampled plots in each dung pile were averaged to obtain one value per distance per block. All evaluated data were transformed using Box-Cox transformations (Zar 1996) to meet ANOVA assumptions. Multiple comparisons were then carried out within the distance treatment using Tukey test with an alpha critical value of 0.05. Beta diversity (Whittaker 1972) and species complementarity (Colwell and Coddington 1994) were calculated for both sets of plots (by the dung piles and 5m away).

Percentage cover was estimated for the following plant groups defined *a priori*: trees and shrubs (woody species), graminoids (grasses and sedges), broad-leaved (dicotyledonous herbs) and exotic species. Percentage cover for each of these groups and percentage cover of bare ground were compared along the two measured gradients using a non-parametric ANOVA in blocks (Friedman test, T², Zar 1996) as the data did not show a normal distribution. Here we also calculated mean values for plots at the same distance from each dung pile, so that a distance value was obtained for each block. Multiple comparisons by pairs were carried out following Conover (1999) to complement the results.

## Results

A total of 98 plant species were recorded during the sampling. Thirty-five of them were present in at least 19 (5%) sampling plots (Table 1). All these more frequent species were recorded at all the measured distances from the dung piles. Some rare plants, like *Rhynchosida physocalyx*, *Cynodon dactylon*, and *Heliotropium amplexicaule* were present only in the immediate vicinity of the piles, while no species were recorded exclusively at 5m.

Percentage of bare ground was significantly reduced with increasing distance from the dung pile edge ( $F_{5, 70}$ = 22.41; p<0.01). Percentage cover of bare soil at the small scale ranged from 23.8% near to the dung piles to 7% 1.5 metres away ( $F_{5, 70}$ = 38.44, p<0.001) (Figure 2). Species richness fluctuated along the smaller gradient and the difference was significant ( $F_{5, 70}$ = 2.42; p<0.05), but Tukey's test only showed significant differences between the second and last plots (7.46 vs 8.56 species per plot respectively), with no

<b>Table 1.</b> List of the most frequent species (frequency ≥5%) and their percentage of appearance at different distances from the dung pile
edge. Abbreviations; BL: broad-leaved species, E: exotic species, GR; graminoids, N: native species; SH: shrubs.

Species	Plant Group	Status	Distance (cm) n=52 for each distance						
			0–25	25–50	50–75	75–100	100- 125	125– 150	500- 600
Adesmia incana	GR	N	9.6	9.6	5.8	11.5	9.6	11.5	9.6
Aristida pallens	GR	N	25.0	28.8	32.7	26.9	44.2	48.1	53.8
Berroa gnaphalioides	BL	N	30.8	28.8	19.2	26.9	30.8	28.8	38.5
Botriochloa laguroides	GR	N	19.2	17.3	15.4	23.1	19.2	11.5	42.3
Carex rupicola	GR	N	19.2	19.2	21.2	28.8	23.1	21.2	32.7
Centaurea calcitrapa	BL	E	13.5	7.7	5.8	3.8	3.8	5.8	1.9
Chaptalia piloselloides	BL	N	26.9	21.2	13.5	26.9	17.3	23.1	46.2
Chevreulia samentosa	BL	N	25.0	28.8	38.5	42.3	36.5	32.7	61.5
Daucus pusillus	BL	N	5.8	5.8	5.8	7.7	5.8	11.5	15.4
Dichondra repens	GR	N	9.6	5.8	15.4	5.8	11.5	11.5	19.2
Dichondra sericea	BL	N	11.5	5.8	7.7	5.8	3.8	5.8	15.4
Echium plantagineum	BL	E	1.9	5.8	7.7	7.7	1.9	7.7	1.9
Eleusine tristachya	GR	N	17.3	11.5	15.4	19.2	13.5	7.7	28.8
Eragrostis lugens	GR	N	36.5	34.6	38.5	36.5	40.4	38.5	50.0
Eryngium nudicaule	BL	N	48.1	48.1	51.9	46.2	40.4	46.2	84.6
Eryngium paniculatum	GR	N	9.6	15.4	9.6	17.3	13.5	11.5	23.1
Evolvulus sericeus	BL	N	21.2	7.7	19.2	23.1	15.4	19.2	38.5
Glandularia spp	BL	N	3.8	3.8	9.6	5.8	9.6	7.7	9.6
Helenium radianum	SH	N	15.4	15.4	25.0	21.2	11.5	23.1	36.5
Juncus microcephalus	GR	N	7.7	7.7	9.6	9.6	11.5	5.8	21.2
Lolium multiflorum	GR	E	7.7	3.8	7.7	5.8	7.7	3.8	3.8
Margyricarpus pinnatus	SH	N	34.6	32.7	30.8	36.5	44.2	40.4	53.8
Mimosa rocae	SH	N	3.8	5.8	7.7	5.8	7.7	7.7	5.8
Paronychia brasiliana	BL	N	13.5	13.5	11.5	11.5	15.4	15.4	30.8
Pavonia cymbalaria	SH	N	42.3	44.2	36.5	48.1	48.1	40.4	61.5
Petrorhagia nanteuilii	BL	E	15.4	11.5	17.3	17.3	21.2	17.3	5.8
Pfaffia gnaphaloides	BL	N	25.0	19.2	30.8	25.0	25.0	26.9	32.7
Piptochaetium haeckelii	GR	N	11.5	13.5	13.5	13.5	13.5	13.5	9.6
Piptochaetium medium	GR	N	26.9	26.9	25.0	28.8	23.1	28.8	32.7
Piptochaetium stipoides	GR	N	90.4	90.4	90.4	86.5	86.5	88.5	86.5
Plantago patagonica	GR	N	15.4	7.7	13.5	17.3	9.6	9.6	17.3
Plantago lanceolata	BL	E	5.8	5.8	1.9	7.7	5.8	3.8	1.9
Plantago myosurus	BL	N	11.5	15.4	11.5	15.4	15.4	23.1	36.5
Sporobolus indicus	GR	N	13.5	9.6	15.4	9.6	5.8	3.8	17.3
Stipa papposa	GR	N	17.3	11.5	15.4	15.4	13.5	15.4	17.3

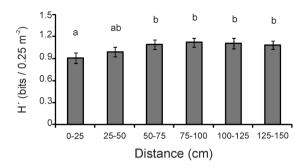
differences among the others. Diversity (i.e. Shannon Index) was low in the plot nearest the dung pile and increased with distance from the pile ( $F_{5,70}$ = 6.54; p<0.01) (Figure 3).

Shrubs was the only plant group that showed differences in cover at the small scale, increasing in abundance further away from the dung piles  $(T^2=5.43, p<0.001; df 14)$  (Figure 4).

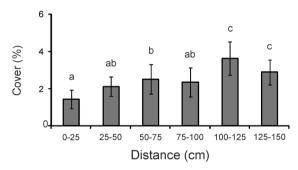
In the case of the comparison between plots on the larger scale, percentage of bare ground was higher in the plots near the dung piles reaching ca. 17.7%, while those placed 5 meters only averaged 3.5% of bare ground ( $F_{1,14}$ = 110.87; p<0.01). Mean species richness of the square metre next to the dung pile was 15.83 species/m<sup>2</sup>, significantly greater than for the plots placed further away (12.19 species/m<sup>2</sup>)

 $(F_{1,14}=12.13; p<0.01)$ . However, the Shannon diversity index did not show any differences at this scale  $(F_{1,14}=0.04; p>0.90)$ . Beta diversity was lower by the dung piles than in the control plots 5 m away (3.73 and 4.25, respectively), and species complementarity between both sampling sets was 41%.

Cover of exotic species was greater in plots near the dung piles, where it reached 1.6%, whereas it was less than 0.4% at five metres from the dung pile edge (T<sup>2</sup>=91; p<0.001; df 14). The rest of the plant groups showed no differences in cover over the larger gradient. Some exotic species, like *Centaurea calcitrapa*, *Lolium multiflorum* and *Petrorhagia nanteuilii* reduced their frequency at greater distances to the dung pile (Table 1).



**Figure 3.** Mean Shannon diversity index (H') at different distances from the dung pile edge along the smaller gradient. Vertical bars represent standard error. Different letters indicate significant differences between the means (Tukey test p < 0.05).



**Figure 4.** Percentage cover of woody species along the smaller gradient. Vertical bars represent standard error. Different letters indicate significant differences between the means, with a probability of global error p<0.05.

### Discussion

Our results evidenced the occurrence of a gradient of vegetation change around dung piles. The percentage of bare ground decreased from the plots next to the dung piles (the first 25 cm) to the greatest distance (at five metres from the dung pile edge). This effect might be due to the intensive use of these areas by the animals (Bouman 1986), causing a considerable loss of plant cover by trampling, or alternatively as an effect of the elevated concentration of solutes in urine and manure.

Shannon diversity increased with distance from the dung piles at the small scale; however, species richness did not vary significantly along the gradient. These changes in diversity could be explained by changes in the relative abundance of the species present, resulting in an increase in plant evenness at greater distances. It is possible that the immediate surroundings of the dung piles contain high levels of nutrients resulting in

the dominance of a small group of tolerant species. However, at the larger scale a greater number of species grew adjacent to the dung piles (1 m² plots) than in the grassland plots five metres away from their edges. The gradient of concentration of nutrients and humidity associated with the immediate surroundings of the dung piles could result in greater species richness as compared to the areas that are further away from the direct influence of the manure as was found by Omaliko (1981) and Dai (2000).

From the point of view of biodiversity conservation in the area, the increase in the frequency and percentage cover of exotic plant species close to the manure heaps is especially interesting. Exotic species often behave as opportunists, taking advantage of increments in the availability of nutrients associated with disturbances (Davis et al. 2000; Lake and Leishman 2004). According to Grime (2001) an increase in the availability of resources may occur in two ways: by a reduction of plant biomass, and so of consumption, or by directly increasing the supply. Both processes might be occurring in the area next to the dung piles, mainly due to the reduction in the abundance of vegetation (high percentages of bare ground) and to the liberation of nutrients from the manure. In this way, the vicinity of dung piles would have resources available for the invaders, making the immediate environment particularly susceptible to invasion (Davis et al. 2000). Some of the exotic species that were recorded in this work also have been cited as growing directly on the dung piles, like in the case of Centaurea calcitrapa (Loydi and Zalba 2009). Others, like Petrorhagia nanteulii, Echium platagineum and Lolium multiflorum, which were present in low abundances in our sampling plots, are highly abundant in the soil seed bank of the study area (Loydi et al. 2012), representing a group of r-selected, ruderal species. All these plants behave as invasives in different regions of Argentina (InBiAr 2013) and in the whole Pampas biome (Fonseca et al. 2013).

The increase observed in the percentage cover of woody plants as the distance from the dung pile edge increases might be due to a behaviour factor of the feral horses that might choose areas without shrubs for depositing their dung. Beever and Brussard (2000) showed that males usually select areas where vegetation is scarce or absent for depositing manure as a function of territory or attraction. Alternatively, a generalized pattern of increase in the cover of shrub species in direct relation with grazing has been shown for the

study area (Barrera 1997: de Villalobos and Zalba 2010) and so the lower abundance of woody plants next to the dung piles might be explained by a lower intensity of grazing in their immediate surroundings. This situation is consistent with the tendency of feral horses to avoid grazing in latrine areas (Shiyomi et al. 1998; Laucougaray et al. 2004; but see also Lamoot et al. 2004). A complementary experiment placing dung heaps in plots with different amounts of vegetation will greatly help to elucidate if the correlation between dung, bare soil and exotic plants is spurious (horses defecate where vegetation is sparse and non-native more easily establish in bare batches) or if there is a real effect among the studied factors.

The results of this study demonstrate that feral horses dung piles are associated with changes in surrounding plant communities, and could result in facilitation effects for the establishment of alien plants, many of them being also probably transported in the manure (Loydi and Zalba 2009). In this way, dung piles should be considered as areas of high priority in strategies of prevention and control of alien plants invasions in natural grasslands.

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