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Glyphosate effects on floristic composition and species diversity in the Flooding Pampa grassland (Argentina)

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ABSTRACT

Temperate grasslands of Argentina are extensively grazed by domestic livestock. Primary production follows a seasonal pattern, with maximum vegetation growth in late spring and minimum in winter. During the last decade, winter forage productivity has been encouraged by promoting the growth of cool-season annual grasses through late summer applications of glyphosate. The aim of this study was to describe structural changes in grassland vegetation associated with glyphosate treatments, as applied in a commercial livestock farm in the Flooding Pampa. Vegetation composition was assessed from spring 2006 to late summer 2008, in three paddocks that had never been exposed to any kind of herbicide, and three other paddocks treated with glyphosate in late summer in the previous 5 years. In the paddocks treated with glyphosate, basal cover of cool-season annual grasses and forage value in spring were greater but basal cover of cool-season perennial grasses, warm-season tussock grasses, warm-season legumes, total basal vegetation cover and forage value in summer were much lower respect to untreated paddocks. The shift in floristic composition resulted in less rich and even assemblages, dominated by an annual species and impoverished in native and perennial species. These structural changes may alter ecosystem processes through the increase of soil salinization and water losses in summer, may affect the seasonal pattern of productivity and may threaten biodiversity conservation and sustainability of wild life.

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1. Introduction

Agricultural intensification over the past 60 years has reduced habitat structure heterogeneity in time and space, resulting in widespread decline in biodiversity (Benton et al., 2003). The changes in land use led to the simplification of complex natural ecosystems and increased input use of agrochemicals (Tscharntke et al., 2005). Herbicides have been widely used to replace vast areas of temperate rangelands by crops throughout the world (Gelbard, 2003), reducing agroecosystems biodiversity (Freemark and Boutin, 1995; McLaughlin and Mineau, 1995; Schütte, 2003). In animal production systems, herbicides are also used to improve forage yield mainly in cultivated pastures or forage crops, but rarely in native grasslands grazed by ungulates. Selective herbicides are applied in North American rangelands to control forbs or shrubs (Ortmann et al., 1998; Fuhlendorf et al., 2002; Kreuter et al., 2005; Monaco et al., 2005; Cummings et al., 2007; Sellers and Mullahey, 2008; Lulow, 2008). In Europe and USA, selective herbicides have been used for conservation and restoration purpose, such as to eradicate exotic and invasive plant species or to reduce competition from dominant plant species in order to increase grassland diversity (Milligan et al., 2003; Mau Crimmins, 2007; Westbury and Dunnett, 2008). In South American grasslands, herbicides are rarely used and their effects on productivity and biodiversity of grasslands are sparsely studied. However in Argentina, herbicide use in humid grassland areas increased in the last decade because of agriculture intensification.

Changes in land use in the Pampas began slowly in the 1960s and this process accelerated at the end of the 1970s (Barsky and Gelman, 2009; Manuel-Navarrete et al., 2009). The most dramatic technological innovation occurred in 1996 with the introduction of genetically modified soybeans tolerant to glyphosate (Trigo and Cap, 2003) and the elimination of soil tillage (no tillage) (Satorre, 2005). Pastures and annual forage crops were replaced by wheat–soybean relay cropping, maize and sunflower crops. As a direct consequence of the reduction of land assigned to forage production in the more fertile area of the Pampa region, the animal stocking rate increased in the less fertile lands, such as Flooding Pampa.

The Flooding Pampa is a 90,000 km^2 area in the eastern portion of Pampa region that extends from 35°S to 38°S and from 58°W to 61°W, where cow–calf operations to produce yearlings has been the main economic activity up to a few years ago. The low fertility of soils and frequent flooding precludes the adoption of cultivated pastures or crops (Soriano et al., 1991). Climatic

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conditions allow the sequential growth of C_3 and C_4 grasses during the cool and warm-season, respectively. However, forage production follows a seasonal pattern, since maximum growth rate (30–80 kg DM ha^{-1} d^{-1}) occurs in late spring and minimum (3–10 kg DM ha^{-1} d^{-1}) in winter (Sala et al., 1981; Oesterheld and León, 1987). The shortage of forage during winter restricts the carrying capacity of this system (Deregibus et al., 1995). To satisfy the higher animal requirement, different practices to increase high quality forage production during winter were developed: (a) applying rotational grazing (Jacobo et al., 2006) that promotes early establishment of Italian ryegrass (*Lolium multiflorum* Lam.) (Jacobo et al., 2000); (b) phosphate fertilization, that increases the productivity of both C_3 annual grasses and legumes (Rodríguez et al., 2007); or (c) nitrogen fertilization (Fernández Greco and Agnusdei, 2004).

In the last decade, the application of glyphosate and nitrogen fertilization has been promoted to increase winter forage productivity in the native grassland of the Flooding Pampa. Glyphosate is sprayed in late summer to eliminate vegetation composed mainly of C_4 grasses and forbs in order to improve germination and establishment of cool-season (C_3) annual grasses, whose major component is *L. multiflorum*. This practice increased winter forage production per hectare and allowed improvement of stocking rate and meat production (Bilello and Zeberio, 2002). However, the application of glyphosate may potentially increase dominance of cool-season annual grasses, reduce the contribution of warm-season species, reduce floristic richness and shift species composition of these grassland plant communities. These structural changes may alter ecosystem functioning and processes, such as the pattern of seasonal productivity or water balance. The aim of this work was to study structural changes associated with the application of glyphosate in grassland communities of the Flooding Pampa, and their implications on biodiversity conservation, ecosystem processes and livestock management. We analyzed structural traits such as basal cover of plant functional groups, total vegetation cover and bare soil, floristic composition, species diversity components and forage quality, surveyed in paddocks never exposed to any kind of agrochemical product and in paddocks treated with glyphosate in late summer in the previous 5 years. Vegetation data were collected from paddocks of a commercial livestock farm representative of the livestock farms of this region during two consecutive growing seasons.

2. Materials and methods

2.1. Study area

The regional climate of Flooding Pampa is temperate, subhumid, with mean annual precipitation varying from 1000 in the north to 850 mm in the south, evenly distributed throughout the year. Monthly temperatures range from 6.8 °C in July–August to 21.8 °C in January. Because of the flat relief, the occurrence of a high water table and the nature of parent material, most soils belong to the halo-hydromorphic complexes and associations influenced by flooding (Natraquolls, Natracualfs, Natralbolls and Argialbolls). Well-drained soils (Hapludols and Argiudolls) are restricted to the highest landscape areas where pastures and crops are cultivated. Vegetation is arranged as a complex mosaic of herbaceous communities mainly determined by landscape features (Soriano et al., 1991; Perelman et al., 2001).

2.2. Sampling sites

Data were collected from a commercial 1600 ha farm located in Azul (Buenos Aires Province), the center of Flooding Pampa region (36°40′S, 59°32′W, 80 m asl.). The main activity of the farm is Angus and Hereford cow–calf operations and calf breeding, grassland being the main forage source. Rotational grazing is performed among 60 ha paddocks.

The six paddocks selected from the farm rotational grazing system were dominated by a humid mesophytic meadow community. Humid mesophytic meadows develop on typical Natraquolls or Natralbolls soils in midland extended slopes (<3%) and have been most intensively exposed to glyphosate treatments. These soils are chararacterized by an acidic, non-saline A_1 horizon and a saline highly alkaline B_2 horizon (Taboada and Lavado, 1988). Dominant species include *L. multiflorum* Lam., *Paspalum dilatatum* Poir., *Bothriochloa laguroides* (DC.) Herter, *Sporobolus indicus* (L.) R. Br., *Panicum milioides* Nees ex Trin., *Nassella neesiana* (Trin. & Rupr.) Barkworth., *Briza subaristata* Lam., *Piptochaetium montevidensis* (Spreng.) Parodi, and *Danthonia montevidensis* Hack. & Arechav. (Perelman et al., 2001).

Three of the six surveyed paddocks have regularly received late summer application of glyphosate during the last 5 years whereas the remaining has never been treated. A rate of 1440 g acid equivalent ha^{-1} of a commercial formulation of glyphosate (Roundup Full II®) with 100 L ha^{-1} water was sprayed in a single application during the first week of March each year. At this time, warm-season species were growing actively and perennial cool-season species were starting their growing period. Glyphosate application was performed with a commercial terrestrial sprayer.

Humid mesophytic meadow communities may respond differently to glyphosate application according to slope position. Along the subtle slope of the humid mesophytic meadows community, the upper or lower topographic position may determine differences of A_1 horizon depth, soil organic matter content (Batista et al., 2005), timing and duration of flooding events (Paruelo and Sala, 1990) and species composition, which may be organized as variants of the same community: the upper and the lower slope position of the landscape (León, 1975). Consequently, sites corresponding to the upper and the lower slope in each paddock were selected to perform the measurements.

2.3. Rainfall and seasonal distribution

Rainfall was recorded monthly at the farm with a Hellman rain gage during the whole experimental period (Fig. 1).

2.4. Vegetation survey

Vegetation was surveyed six times: spring (October), summer (February) and late summer (April) of two consecutive growing season (25 October 2006, 3 February 2007, 2 April 2007, 27 October 2007, 2 February 2008 and 30 March 2008). Plant basal cover and species composition were estimated by the step-point method (Mueller-Dombois and Ellenberg, 1974) along five 10-m long transects (200 points per transect) which were randomly placed in sites corresponding to the upper and lower slope in each paddock. Bare soil, litter or standing dead material were recorded where no living plants were intercepted.

2.5. Species diversity

Species diversity was characterized by its two components: richness and evenness/dominance. Species richness (S) was estimated as the total number of species per paddock. Evenness/dominance was estimated by the Berger–Parker index (d) because it is a simple and easily interpretable measure of dominance. It expresses the proportional abundance of the most abundant species: $d = N_{max}/N$, where N_{max} is the number of individuals of the most abundant species and N is the total number

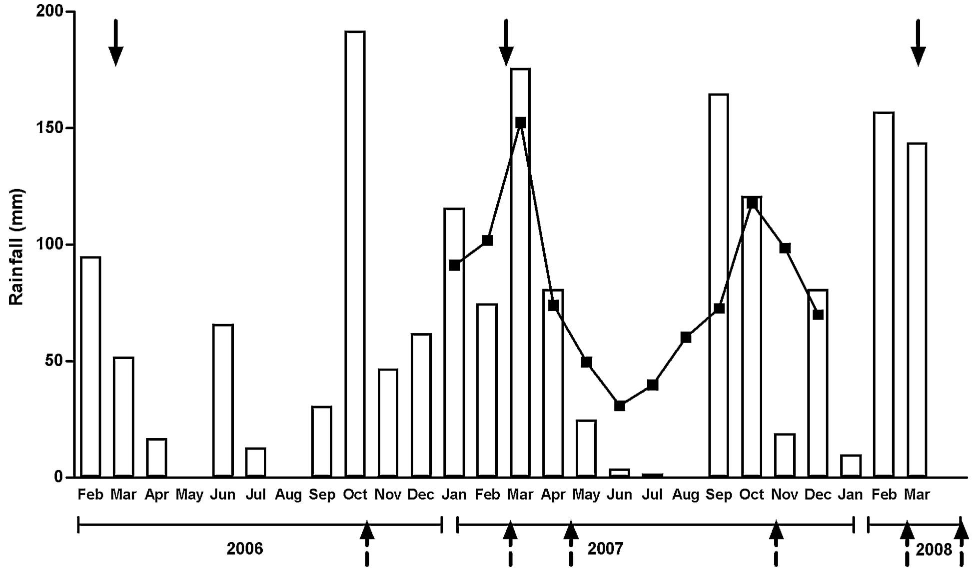

Fig. 1. Monthly rainfall (mm) recorded during the experimental period (bars) and average 1999–2008 monthly rainfall (mm) registered in the commercial livestock farm (line). Full arrows indicate the timing of glyphosate application and dotted arrows indicate vegetation sampling dates.

of individuals. This index ranges between 1 and 0 and the value increases as the assemblage becomes dominated by fewer species with higher abundance. Measures that consider species abundance are known as species diversity indices. We selected the Gini–Simpson's index: $1 - D = 1 - \sum p_i{}^2$, where p is the proportion of each species in each paddock, because it provides a good estimate of diversity at relatively small sample sizes and is easily interpretable. It ranges between 1 and 0 and the value of this measure increases as the assemblage becomes more even and rich. (Magurran, 2004).

2.6. Plant functional groups

Plant species were classified using a hierarchical approach (Lavorel et al., 1997) according to growth form (grasses, dicotyledonous species, sedges), life history (annual, perennial), photosynthetic pathway (C_3, C_4), morphology (tussock, creeping), symbiotic nitrogen fixation (legumes, dicotyledonous forbs) and growing season (cool or warm-season). The following functional groups were thus obtained: cool-season (C_3) annual grasses, cool-season (C_3) perennial grasses, warm-season (C_4) tussock grasses, warm-season (C_4) creeping grasses, cool-season legumes, warm-season legumes, cool-season forbs and warm-season forbs. Relative cover (%) was calculated from total basal cover, including litter, bare soil and standing dead material.

2.7. Forage quality

A forage quality index (FQI) was estimated for each sampling date to determine changes in forage quality associated with glyphosate application. The FQI was calculated using the following equation proposed by Daget and Poissonnet, 1971: $\text{FQI} = \sum p_i \times q_i$, where p_i is the proportion of each species in the sample and q_i is each species specific quality value. q_i values for Flooding Pampa grassland species were proposed by Cahuepe et al. (1985), ranging from 0 (without any forage quality) to 5 (excellent forage quality).

2.8. Statistical analysis

Repeated-measures analysis of variance was performed to evaluate differences in total basal cover and forage quality index (FQI), considering glyphosate treatments as main effect and sampling dates as within-subject effect. When interaction (treatment × sampling date) was statistically significant ($P < 0.05$), we performed analysis of variance to separate treatment means within sampling date.

Treatment effects on basal cover of each functional group, were estimated using data from the sampling date within each growing season at which each group showed its maximum growth: spring (October) for the four cool-season groups and summer (February) for the four warm-season groups, in order to avoid confounded effects between functional group growing season and treatment. A similar procedure was used to compare species diversity indices, using data corresponding to the sampling date when maximum values of these variables were found for each growing season. Repeated-measures analysis of variance for each functional group and species diversity indices was performed, considering glyphosate treatments as main effect and growing seasons as within-subject effect. When interaction (treatment × sampling date, treatment × growing season) were statistically significant ($P < 0.05$), we performed analysis of variance to separate treatment means within sampling date or growing season

In order to describe the variation in species composition between treatments and growing season, ordination and classification multivariate techniques excluding rare species (constancy lower than 15%) were performed. Correspondence analysis (Greenacre, 1984) using frequency data of species in each sample units (paddocks) was carried out in order to describe the major sources of variation in species composition. We performed a multi-response permutation procedure to test multivariate differences between untreated paddocks and glyphosate treated paddocks. To identify particular species responsible for differences between these two groups, Indicator Species Analysis and Monte Carlo test were performed.

The Statistica software (StatSoft, Inc.) was used to perform parametric univariate analysis and PC-ORD™ version 4 software (MjM Software, Inc.) was used for multivariate analysis.

3. Results

3.1. Total basal vegetation cover

Total basal vegetation cover surveyed in summer in glyphosate treated paddocks was 6-fold lower in the upper slope (Fig. 2a)

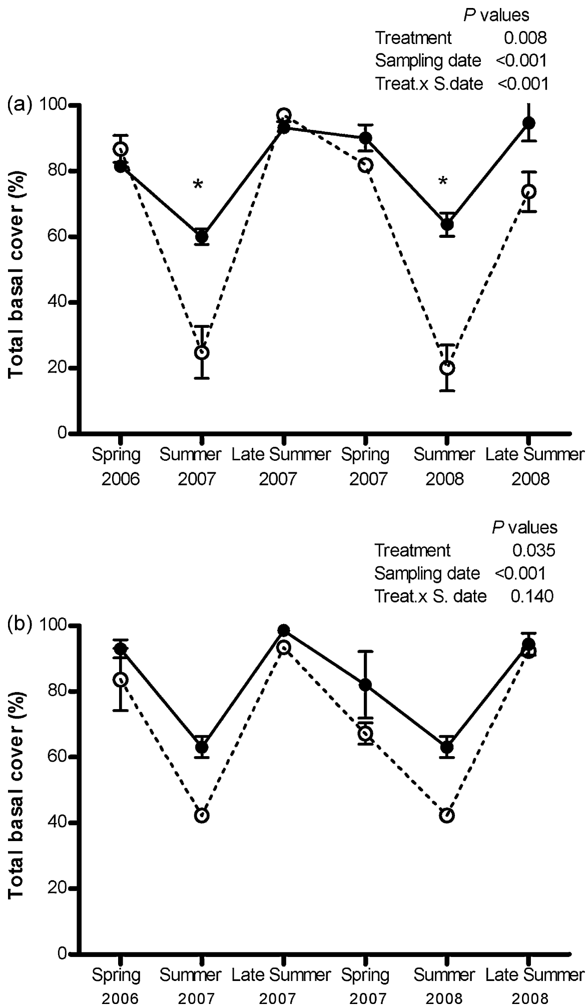

Fig. 2. Total basal vegetation cover in the upper (a) and the lower (b) slope position occurring in paddocks untreated (full line) and treated with late summer applications of glyphosate (dotted line) in the 2006/2007 and 2007/2008 growing seasons. Vertical lines indicate standard error of the mean. Insert: *P*-values of the repeated-measures analysis of variance. Asterisks indicate treatments differ within recording date.

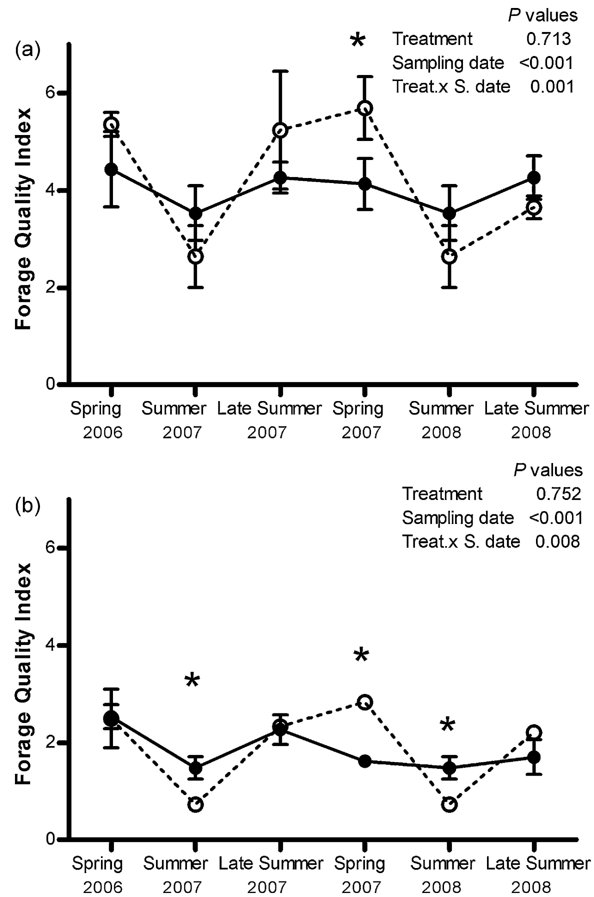

Fig. 3. Forage Quality Index in the upper (a) and the lower (b) slope position occurring in paddocks untreated (full line) and treated with late summer applications of glyphosate (dotted line) in the 2006/2007 and 2007/2008 growing seasons. Vertical lines indicate standard error of the mean. Insert: *P*-values of the repeated-measures analysis of variance. Asterisks indicate treatments differ within recording date.

and 1.5-fold lower in the lower slope (Fig. 2b) than in untreated paddocks. As the proportion of litter plus standing dead material achieved less than 10% throughout all paddocks and sampling dates (data not shown), the proportion of bare soil was well represented by the inverse of total basal vegetation cover.

3.2. *Forage quality*

Forage quality was greater in glyphosate treated paddocks than in untreated paddocks only in spring 2007 in the upper and the lower slope position (Fig. 3). In summer 2007 and 2008, forage quality in glyphosate treated paddocks in the lower slope position was 2-fold lower than in untreated paddocks (Fig. 3b). In the upper slope position, forage quality in summer was similar in glyphosate treated and untreated paddocks (Fig. 3a).

3.3. *Functional groups basal cover*

Basal cover of cool-season annual grasses in glyphosate treated paddocks was 5–6-fold greater in the upper slope (Table 1a) and 2–5-fold greater in the lower slope than in untreated paddocks (Table 1b). Conversely, basal cover of cool-season perennial grasses, warm-season tussock grasses, warm-season legumes and sedges in glyphosate treated paddocks was lower than in untreated paddocks in the upper and the lower slope position (Table 1). Basal cover of cool-season legumes and cool and warm-season dicotyledonous forbs was similar in glyphosate treated and untreated paddocks in both slope position (Table 1). Basal cover of warm-season creeping grasses in the lower slope position was higher in glyphosate treated paddocks than in untreated paddocks (Table 1b), while in the upper slope position, basal cover in glyphosate treated and untreated paddocks was similar (Table 1a). Only legumes showed differences among growing seasons in the upper slope. Basal cover of cool-season legumes surveyed in spring 2007 was higher than that surveyed in spring 2006 (Table 1a), while rainfall in September 2007 was higher than that in September 2006 (Fig. 1). Basal cover of warm-season legumes in untreated paddocks surveyed in summer 2008 was lower than that surveyed in summer 2007 (Table 1a), while rainfall in January 2008 was much lower than that in January 2007 (Fig. 1).

3.4. *Species diversity*

Species richness was 2–3-fold lower in glyphosate treated paddocks than in untreated paddocks in both slope positions (Table 2). The dominance index was 2–3-fold higher in glyphosate treated paddocks than in untreated paddocks (Table 2). Taking into account the relative abundance of all species recorded in each paddock, the diversity index was lower in glyphosate treated paddocks than in untreated paddocks (Table 2). Therefore, assemblages of species from glyphosate treated paddocks were less rich and even, domi-

Table 1
Basal cover (%) of functional groups for the upper (a) and lower (b) slope position from untreated and glyphosate treated paddocks in the 2006/2007 and 2007/2008 growing seasons.

(a) Upper slope position

Growing season	2006/2007		2007/2008			*F*-ratio	df	*P*-value
Treatment	Untreated	Glyphosate	Untreated	Glyphosate				
Cool-season annual grasses	9.6 (3.2)	51.6 (10.7)	9.0 (4.5)	64.2 (4.9)	*T*	125.1	1	<0.01
					GS	0.5	1	0.5
					GS × *T*	0.7	1	0.5
Cool-season perennial grasses	28.2 (4.1)	0.8 (0.8)	27.3 (3.2)	0.6 (0.5)	*T*	59.5	1	<0.01
					GS	0.1	1	0.7
					GS × *T*	0.0	1	0.8
Cool-season legumes	0.6 (0.3)	3.8 (2.4)	9.2 (2.7)	11.9 (2.8)	*T*	1.2	1	0.3
					GS	19.9	1	<0.05
					GS × *T*	0.0	1	0.9
Cool-season dicotyledonous forbs	12.2 (5.5)	9.4 (5.3)	28.2 (4.8)	5.2 (1.0)	*T*	7.0	1	0.1
					GS	2.0	1	0.2
					GS × *T*	5.9	1	0.1
Warm-season tussock grasses	36.7 (2.4)	8.9 (4.0)	28.1 (6.3)	1.0 (1.0)	*T*	75.3	1	<0.01
					GS	3.2	1	0.1
					GS × *T*	0.0	1	0.9
Warm-season creeping grasses	8.8 (5.1)	2.8 (2.8)	5.0 (5.0)	3.1 (2.0)	*T*	0.6	1	0.5
					GS	0.6	1	0.5
					GS × *T*	0.8	1	0.4
Warm-season legumes	14.7 (1.4)	1.7 (1.2)	4.3 (0.3)	0.6 (0.3)	*T*	80.8	1	<0.01
					GS	34.4	1	<0.01
					GS × *T*	22.0	1	<0.01
Warm-season dicotyledonous forbs	22.7 (8.9)	18.3 (7.7)	26.7 (6.9)	23.9 (8.5)	*T*	0.2	1	0.7
					GS	0.4	1	0.6
					GS × *T*	0.0	1	0.9
Sedges	1.9 (0.3)	0.6 (0.3)	1.6 (0.2)	0.6 (0.3)	*T*	10.9	1	<0.05
					GS	1.5	1	0.3
					GS × *T*	0.7	1	0.4

(b) Lower slope position

Growing season	2006/2007		2007/2008			*F*-ratio	df	*P*-value
Treatment	Untreated	Glyphosate	Untreated	Glyphosate				
Cool-season annual grasses	13.2 (3.8)	30.8 (17.3)	8.6 (4.5)	43.1 (9.7)	*T*	7.6	1	0.1
					GS	0.1	1	0.7
					GS × *T*	0.6	1	0.5
Cool-season perennial grasses	16.8 (5.4)	2.0 (1.7)	19.6 (1.1)	5.0 (3.5)	*T*	33.9	1	<0.01
					GS	0.5	1	0.5
					GS × *T*	0.0	1	0.9
Cool-season legumes	7.9 (2.1)	1.1 (1.1)	4.1 (2.2)	3.8 (1.4)	*T*	2.7	1	0.2
					GS	0.2	1	0.7
					GS × *T*	6.7	1	0.1
Cool-season dicotyledonous forbs	11.9 (5.1)	11.9 (6.8)	22.5 (6.5)	8.8 (7.4)	*T*	1.2	1	0.3
					GS	0.3	1	0.6
					GS × *T*	0.9	1	0.4
Warm-season tussock grasses	14.8 (2.8)	3.4 (1.9)	24.8 (7.4)	6.4 (0.9)	*T*	145.3	1	<0.01
					GS	6.4	1	0.1
					GS × *T*	1.9	1	0.3
Warm-season creeping grasses	13.3 (4.6)	42.0 (4.6)	6.3 (1.5)	18.6 (3.9)	*T*	27.9	1	<0.01
					GS	15.2	1	<0.05
					GS × *T*	4.4	1	0.1
Warm-season legumes	18.2 (4.4)	6.2 (4.8)	8.8 (2.8)	0.7 (0.1)	*T*	9.4	1	<0.05
					GS	3.8	1	0.1
					GS × *T*	0.3	1	0.6
Warm-season dicotyledonous forbs	31.4 (4.5)	17.1 (10.2)	26.8 (4.7)	25.7 (3.8)	*T*	1.5	1	0.2
					GS	0.1	1	0.7
					GS × *T*	1.1	1	0.3
Sedges	13.2 (3.8)	30.8 (17.4)	8.7 (4.5)	43.1 (9.7)	*T*	7.6	1	0.1
					GS	0.1	1	0.7
					GS × *T*	0.6	1	0.5

Values are means of paddocks and standard errors of means are shown within parentheses. *F*-ratio, degree of freedom (df) and *P*-value resulting of the repeated-measures analysis are indicated (*T*, treatments; GS, growing season; GS × *T*, interaction among treatments and growing season).

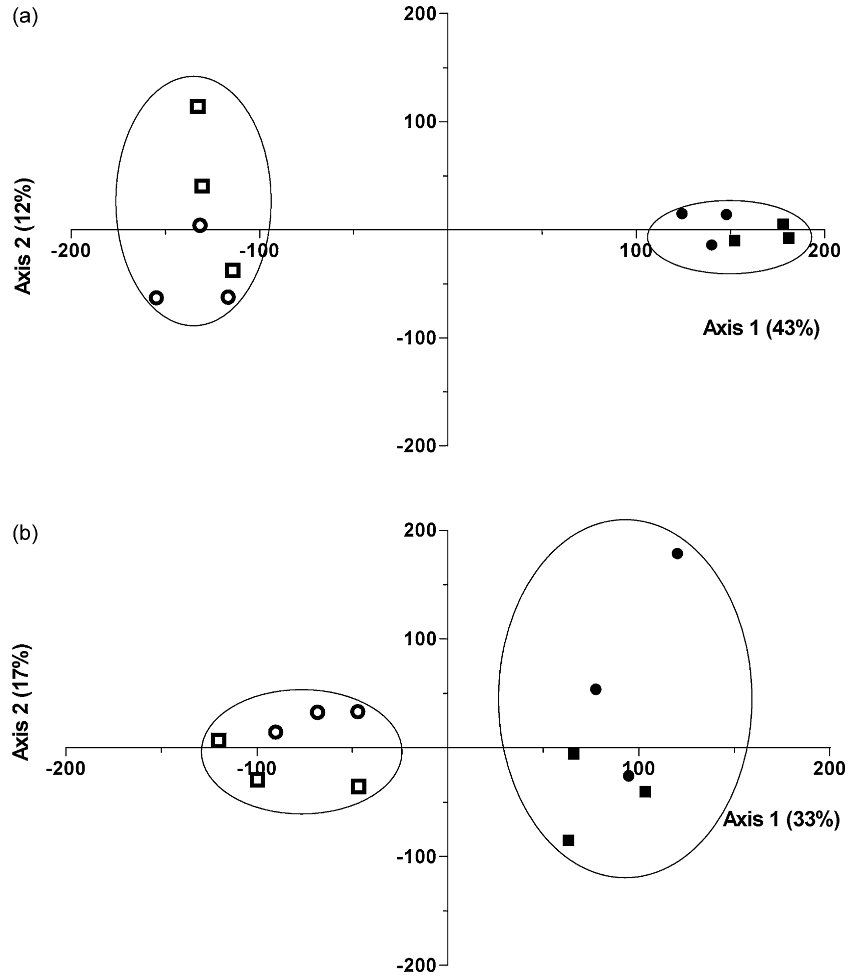

Fig. 4. Ordination of vegetation surveys corresponding to untreated (empty symbols) and glyphosate treated (black symbols) paddocks of the upper (a) and lower (b) slope position in the 2006/2007 (circle) and 2007/2008 (square) growing seasons. Ellipsoids represent groups derived from MRPP procedure.

nated by one species, *L. multiflorum*, with high abundance (32–45%) and consequently, less diverse than assemblages from untreated paddocks.

3.5. Floristic composition

The ordination of species surveyed in the upper and in the lower slope position showed that the principal gradient of floristic composition was glyphosate treatment (Fig. 4). In the upper slope position, the first two axes of correspondence analysis accounted for 55% of total variance (Fig. 4a). The first axis accounted for 43% of total variance and reflected a shift in species composition in glyphosate treated paddocks respect to untreated paddocks. The second axis accounted for 12% of total variance and suggested a gradient associated with increasing proportion of *N. neesiana* in untreated paddocks. MRPP procedure confirmed the difference between glyphosate treated paddocks group and untreated paddocks group ($P < 0.001$), with a similar average distance among members of each group (30 and 32, respectively). Species responsible of differences between glyphosate treated and untreated paddocks groups were identified through Indicator Species Analysis and Monte Carlo test, resulting *L. multiflorum*, *Bromus catarthicus* Vahl, *Schedonorus arundinaceus* (Schreb.) Dumort., *N. neesiana*, *P. dilatatum* Poir., *B. laguroides* (DC.) Herter, *S. indicus* (L.) R. Br., *Lotus glaber* Mill., *Phyla canescens* (Kunth) Greene, *Ambrosia tenuifolia* Spreng. and *Juncus imbricatus* Laharpe (Appendix 1).

In the lower slope position, the first two axes of correspondence analysis account for 50% of total variance (Fig. 4b). The first axis accounted for 35% of total variance and also reflected a shift in species composition in glyphosate treated paddocks respect to untreated paddocks. The second axis accounted for 17% of total variance and showed a higher heterogeneity among species composition of glyphosate treated paddocks than among untreated paddocks. MRPP procedure confirmed the difference between glyphosate treated paddocks and untreated paddocks groups ($P = 0.002$) and a greater heterogeneity of floristic composition in glyphosate treated paddocks because average distance among members of this group was greater than the average distance among members of untreated paddocks (39 and 24, respectively) (Fig. 4b). Species responsible of differences between glyphosate treated and untreated paddocks were *S. arundinaceus*, *P. dilatatum*, *Glyceria multiflora* Steud., *Panicum bergii* Arechav., *Cynodon dactylon* (L.) Pers., *Distichlis spicata* (L.) Greene, *Medicago lupulina* L., *L. glaber*, *Alternanthera philoxeroides* (Mart.) Griseb, *P. canescens* and *Oxalis articulata* Savigny (Appendix 1). In this slope position, *L. multiflorum* was not responsible of differences between glyphosate treated and untreated paddocks group which suggests a erratic response to the application of the herbicide.

In the upper slope position, 36 species were not recorded in the glyphosate treated paddocks: 23 of them were native species and within them, 13 were native grasses. In the lower slope, 17 species were not recorded in the glyphosate treated paddocks: 16 of

Table 2
Diversity indices for the upper (a) and lower (b) slope position from untreated and glyphosate treated paddocks in the 2006/2007 and 2007/2008 growing seasons.

a) Upper slope position

Growing season	2006/2007		2007/2008			F-ratio	df	P-value
Treatment	Untreated	Glyphosate	Untreated	Glyphosate				
Richness (S)	55 (2.6)	20 (2.1)	54 (1.0)	16 (1.202)	T	365.6	1	<0.01
					GS	1.7	1	0.3
					GS × T	0.5	1	0.5
Dominance (Berger–Parker's index, d)	0.22 (0.01)	0.38 (0.05)	0.14 (0.03)	0.5 (0.07)	T	67.7	1	<0.01
					GS	0.0	1	1.0
					GS × T	2.0	1	0.2
Diversity (Gini–Simpson's index, 1 − D)	0.90 (0.01)	0.78 (0.03)	0.93 (0.01)	0.71 (0.04)	T	150.7	1	<0.01
					GS	0.4	1	0.5
					GS × T	3.6	1	0.1

b) Lower slope position

Growing season	2006/2007		2007/2008			F-ratio	df	P-value
Treatment	Untreated	Glyphosate	Untreated	Glyphosate				
Richness (S)	33 (0.9)	17 (5.2)	35 (1.5)	19 (1.8)	T	49.8	1	<0.01
					GS	0.3	1	0.6
					GS × T	0.0	1	1.0
Dominance (Berger–Parker's index, d)	0.17 (0.02)	0.42 (0.09)	0.123 (0.02)	0.32 (0.06)	T	14.4	1	<0.05
					GS	1.8	1	0.2
					GS × T	0.2	1	0.7
Diversity (Gini-Simpson's index, 1 − D)	0.93 (0.01)	0.76 (0.12)	0.95 (0.01)	0.84 (0.07)	T	14.6	1	<0.05
					GS	1.2	1	0.3
					GS × T	0.6	1	0.5

Values are means of paddocks and standard errors of means are shown within parentheses. Richness (*S*) is the total number of species; Berger–Parker's dominance index (*d*) is the number of individuals of the most abundant species relative to the total number of individuals and Gini–Simpson's diversity index is the complement of the summation of the quadratic value of each species' relative proportion. *F*-ratio, degree of freedom (df) and *P*-value resulting of the repeated-measures analysis for each index are indicated (*T*, treatments; GS, growing season; GS × *T*, interaction among treatments and growing season).

them were native species and, within them, 10 were native grasses. Most dicotyledonous forbs species registered in glyphosate treated paddocks in the upper and the lower slope position were annuals (Appendix 1). The average number of species in glyphosate treated and untreated paddocks of the upper slope position (*S*, Table 2a) was similar to the sum of the species number recorded in each paddock (Appendix 1a). Conversely, in the lower slope position, the average number of species in glyphosate treated and in untreated paddocks (*S*, Table 2 b) was lower than the sum of the species number recorded in each paddock (Appendix 1b). Therefore, floristic composition heterogeneity was greater among paddocks in the lower slope position than among paddocks in the upper slope position.

4. Discussion

Late summer applications of glyphosate increased forage supply in terms of quantity and quality during winter and spring through a higher contribution of cool-season annual grasses, mainly *L. multiflorum*. However, this practice strongly affects the plant community structure of the humid mesophytic meadows of the Flooding Pampa because it produces a significant shift of floristic composition and reduced species richness and diversity. These structural changes may alter ecosystem processes and have important implications for biodiversity conservation and livestock management in the last semi-natural habitats in the Pampas grasslands.

Our results indicated that plant community structure was mainly affected by glyphosate application, while fewer differences in structural traits between slope positions and negligible differences derived from inter-annual rainfall variation were found.

Plant community structure was affected by glyphosate, through direct and indirect effects. Direct effects will vary according to species tolerance and physiological plant activity at the time of application. Indirect effects may occur when functional groups, that are either tolerant or have avoided herbicide application, compete with other functional groups. Growth of plants affected by the herbicide may be further compromised by grazing. Basal cover for cool-season annual grasses, dominated by *L. multiflorum*, was greater in glyphosate treated paddocks. The removal of vegetation cover through heavy grazing or clipping can promote *L. multiflorum* germination by increasing the red–far red ratio for light reaching the soil surface (Deregibus et al., 1994; Rodríguez et al., 1998) and the absence of competition also ensures seedling establishment and plant growth (Jacobo et al., 2000). The practice of spraying glyphosate in late summer removes green vegetation cover. This cause similar effects than heavy grazing or clipping: the promotion of seed germination and seedling growth of *L. multiflorum* during autumn. As an indirect effect of this practice, the great dominance of *L. multiflorum* may cause a strong competition over other functional groups. During late summer, when glyphosate is applied, cool-season perennial grasses, warm-season tussock grasses and warm-season legumes are growing actively, so they are directly affected by glyphosate. Species of these functional groups are more susceptible to this herbicide action than others slow growing species as translocation rate of this systemic herbicide within the plant is higher at higher growth rate (Tworkoski and Sterrett, 1987) These functional groups are also indirectly affected by competition from cool-season annual grasses (Jacobo et al., 2000, 2006). In addition, most perennial grasses (cool- and warm-season tussock species) have low seed production capacity (Bogdan, 1991; Bewley and Black, 1994; Campbell, 1999; Ferrari and López, 2000); therefore, recovery of population density could be delayed. Particularly, *P. dilatatum*, the major component of warm-season tussock grasses, is extremely sensitive to glyphosate (Manuja et al., 2005) and is highly preferred by livestock (Lemcoff et al., 1978). The combination of several years of direct herbicide application, selective grazing and low seed production may cause a great reduction of this plant population. In the case of warm-season legume group, *L. glaber* has a very high seed production capacity (Montes, 1988) but glyphosate application occurs during the seed production period. Therefore, other direct effect of glyphosate may be the gradual reduction of *L. glaber* seed bank. Consistent with our results, biomass of warm-season grasses and *L. glaber* were lower in sites treated with glyphosate of a similar grassland community, respect to untreated sites (Arzadun and Mestelan, 2009). Basal cover for cool-season legumes recorded in glyphosate treated and untreated paddocks was similar, probably because these species have very low growth rate during summer, so direct action of this herbicide may have been negligible. The major member of this group, *T. repens*, exhibits a high seed production rate (Scheneiter, 2001). Although a summer glyphosate application could eliminate some growing plants, *T. repens* seeds dispersed in late spring and early summer may ensure its persistence. Also basal cover for warm-season creeping grasses recorded in glyphosate treated and untreated paddocks was similar. This response may be explained by direct and indirect effects; the tolerance of the main component of this group, *C. dactylon*, to glyphosate (Bedmar, 1992; Dinelli, 2000; Mau Crimmins, 2007), the absence of competition of warm-season tussock grasses and its ability to avoid grazing.

Several differences in structural responses between upper and lower slope position were found. Although basal cover of cool-season annual grasses in untreated paddocks was similar (around 10%) in both slope positions, it increased to a lower extent in the lower slope position in glyphosate treated paddocks. This differential response is attributed to soil and topographic characteristics: a shallower A_1 horizon (>12 cm depth) with lower organic matter content and lower water holding capacity than in the upper slope position (Batista et al., 2005). These environmental conditions are more restrictive for growth of cool-season annual grasses and lead to erratic recruitment of this functional group in glyphosate treated paddocks. Probably because of the weaker competition exerted by this group, basal cover of warm-season creeping grasses was greater in glyphosate treated paddocks. This erratic response to the practice in the lower slope position may be also associated to the greater heterogeneity in floristic composition among paddocks.

Inter-annual rainfall variation had low significance in explaining the response of functional groups and species composition, in spite of the difference registered in rainfall patterns among growing seasons. Conversely, Fuhlendorf et al. (2009) found that the variation in cover of forbs and grasses was better explained by the inter-annual rainfall variation than by the effect of the mix of herbicides 2,4-D and Picloram in the central Great Plains of North America. In our research, only legumes in the upper slope were affected by growing season because both *T. repens* and *L. glaber* are highly dependent on water availability (Scheneiter, 2001; Vignolio and Fernández, 2006). Our results suggest that these legumes would respond to precipitation in the month prior to the recording date.

Floristic richness was much lower and dominance was greater in glyphosate treated paddocks, resulting in a less rich and even community dominated by *L. multiflorum*, which achieved a basal cover of 40–50%. The number of native species surveyed in untreated paddocks from the upper and lower slope position was 32 and 33, respectively, while a much lower number, 8 and 17, of native species was recorded in glyphosate treated paddocks. More than a half of native species not recorded in glyphosate treated paddocks were grasses. Almost all dicotyledonous forbs species not recorded in glyphosate treated paddocks were perennials. This response suggests that some perennial dicotyledonous forbs were directly damaged by glyphosate, which allowed an increase in annual species basal cover. A similar pattern of species shift was found in soybean weed communities in the Rolling Pampas, where floristic diversity decreased and a greater proportion of annual species was registered over time after the introduction of no tillage cropping and soybean transgenic cultivars resistant to glyphosate (de la Fuente et al., 2006). Therefore, the shift in floristic composition led to less rich and diverse communities dominated by annuals and exotic species. These results may have negative implications for biodiversity conservation because of the reduction of floristic richness and native species, which, in turn may affect sustainability of wild life such as soil organisms, insects, arthropods and vertebrates (Freemark and Boutin, 1995). Furthermore, in a lower diverse community dominated by an annual species, resistance and resilience to any kind of disturb (severe droughts, flooding, pests) may be reduced (Grime, 1998).

Our results may also have implication on ecosystem processes. The proportion of bare soil surveyed in summer was greater in glyphosate treated paddocks. Bare soil in summer, when water deficits occurs, promotes soil salinization because soluble salts in the B horizon moves upward throughout the soil profile (Lavado and Taboada, 1987). This process may be more intense in the lower slope position because of the shallower A horizon. The higher basal cover of *D. spicata*, a well-adapted species to saline habitats (Batista et al., 2005) recorded in glyphosate treated paddocks support this idea. Cattle trampling, mainly on bare soil, negatively affect soil structure through the increase of bulk density, causing a reduction of water infiltration (Taboada et al., 1999). Additionally, the lower vegetation cover in summer may determine water losses by evaporation (Lavado and Taboada, 1987) affecting water balance. Ecosystem functioning may also be altered, considering that the shift in species composition determined a concomitant change in the seasonal pattern of productivity: higher winter–spring productivity but much lower summer productivity may be expected in glyphosate treated paddocks.

Our results also have implications for livestock management. The higher contribution of *L. multiflorum* in spring explained the greater forage quality of the grassland community in this season. However, these paddocks showed lower vegetation cover and forage quality in summer, mainly due to the lower contribution of warm-season tussock grasses. Species of this group that account for the differences between glyphosate treated and untreated paddocks were perennial natives: *P. dilatatum*, *B. laguroides*, *S. indicus*, *G. multiflora* and *P. bergii*. These species provide the bulk of forage in summer and autumn (Deregibus et al., 1995) and the main components *P. dilatatum* and *B. laguroides*, have high forage quality (Cahuepe et al., 1985; Jacobo et al., 2006). The reduction of the warm-season legume *L. glaber* in glyphosate treated paddocks may have also contributed to decrease forage quality in summer, considering that this group supplies high protein content that enhances diet quality for livestock. Therefore, the higher forage supply in quantity and quality obtained in winter and spring is counterbalanced by the reduction in summer in glyphosate treated paddocks. This may explain the trend to increase soil surface cultivated with summer forage crops in the last years in the Flooding Pampa region. Other ecologically and economically sustainable practices can improve winter forage production in this region, such as rotational grazing that promotes early establishment of *L. multiflorum* Lam. (Jacobo et al., 2000), or via phosphate fertilization, that increases C_3 annual grasses and legumes productivity (Rodríguez et al., 2007). Both alternatives reduced seasonal variability of plant production by increasing winter forage production, and also improved rangeland condition and carrying capacity (from 0.6 to1.0 animal unit ha^{-1}) because they increased the proportion of high forage value species, reduced the cover of low forage value species and maintained a high floristic diversity (Jacobo et al., 2006). Forage productivity was similar to that obtained with herbicide, without compromising the ecological sustainability of the grassland.

5. Conclusions

This study demonstrates that the practice of applying glyphosate in late summer to increase forage supply during winter and spring has several negative consequences for biodiversity conservation, ecosystem functioning and livestock management in the last semi-natural habitats in the Pampas grasslands. This practice produces a shift of floristic composition that result in less rich and even assemblages, dominated by an annual species and impoverished in native and perennial species. These structural changes may alter ecosystem processes through the increase of soil salinization and water losses during summer, may affect ecosystem functioning, such as the seasonal pattern of productivity and may threaten biodiversity conservation and sustainability of wild life. In order to maximize livestock production in this region is necessary to increase forage supply in winter. Several management practices have demonstrated their effectiveness for this purpose without affecting biodiversity and ecosystem processes (Jacobo et al., 2000; Rodríguez et al., 2007).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.agee.2010.05.003.

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