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Glyphosate effects on seed bank and vegetation composition of temperate grasslands

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Conservation; Disturbance; Diversity; Flooding Pampa grassland; Floristic composition; Herbicide

Nomenclature
Flora del Cono Sur Database from Instituto de Botánica Darwinion (2011)

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Abstract

Questions: Intensification of livestock production has led to the increasing input of agrochemicals, mostly herbicides, in natural and semi-natural grasslands. Glyphosate application shifts vegetation composition of grasslands of the Flooding Pampa region. Consequently, we asked two questions: (1) does the application of glyphosate affect the size, composition and diversity of the seed bank of these grasslands; and (2) to what extent does seed bank determine the vegetation composition of grassland communities affected by glyphosate application?

Location: Temperate-humid grassland of Flooding Pampa region, Buenos Aires, Argentina.

Method: We studied paddocks dominated by native grassland on a commercial livestock farm. Glyphosate is usually applied in late summer to promote winter annual grasses in native grasslands. We chose three paddocks that had never been exposed to any herbicide and three paddocks that had been treated with glyphosate in late summer during the previous 5 years. We extracted soil cores to evaluate seed bank composition from 2007 to 2009 and recorded basal cover of above-ground vegetation from 2008 to 2010 from the upper and the lower position of each paddock.

Results: Glyphosate treatment caused a dramatic shift in seed bank composition, so that seed densities of cool-season annual grasses increased while cool and warm-season perennial grasses, sedges, legumes and dicotyledonous herbs decreased. Richness and diversity of the seed bank were lower and dominance was higher under the glyphosate treatment. Similarity between the seed bank and floristic composition was higher in glyphosate-treated paddocks in the upper position. Strong correspondences between the seed densities of cool-season annual grasses and species composition of glyphosate-treated paddocks and between the seed densities of cool- and warm-season perennial grasses and legumes with the species composition of untreated paddocks were found.

Conclusions: Because the seed bank changes in response to glyphosate treatment involved the local extinction of several native perennial species, community restoration of these grasslands would depend mainly on propagule dispersal from adjacent areas. Our results raise awareness of the risks of widespread herbicide application for biodiversity conservation in the last semi-natural habitats in the Pampas grasslands.

Introduction

The expansion and intensification of agriculture over the past 60 years has caused a widespread reduction in the diversity of species and habitats across landscapes (Benton et al. 2003). This process can be attributed at least partially to the increasing use of agrochemicals (Tscharntke et al. 2005), especially herbicides. Herbicides have been widely used on temperate rangelands converted to croplands throughout the world (Gelbard 2003) and have reduced the biodiversity of agroecosystems (Freemark & Boutin 1995; McLaughlin & Mineau 1995; Schütte 2003). In natural or semi-natural grasslands extensively grazed by domestic ungulates, the application of herbicide is less frequent. Most studies on grasslands have evaluated the effectiveness of selective herbicides on target plant populations (Ortmann et al. 1998; Fuhlendorf et al. 2002; Milligan et al. 2003; Kreuter et al. 2005; Monaco et al. 2005; Cummings et al. 2007; Lulow 2008; Sellers & Mullahey 2008; Westbury & Dunnett 2008). Non-selective systemic herbicides also have been applied to recover grassland communities on abandoned agricultural fields dominated by invasive species. Repeated applications of glyphosate were effective for controlling the invasive grass *Cynodon dactylon* after cessation of cultivation in Arizona (USA), but the contribution of other exotic weeds increased (Mau Crimmins 2007).

With the aim of increasing winter forage production for livestock grazing, late summer applications of glyphosate in native grasslands of the Pampa Region (Argentina) are used. Late summer glyphosate application increased the abundance of *Lolium multiflorum* Lam., a non-native winter annual grass, reduced total annual forage yield (Arzadun & Mestelan 2009), and caused dramatic shifts in seasonal floristic composition (Rodriguez & Jacobo 2010). After glyphosate application, species compositions were less rich and even, and dominated by annual non-native species and not by native perennial species, so that biodiversity conservation was negatively impacted (Rodriguez & Jacobo 2010). *Lolium multiflorum* provides high-quality forage during winter, a critical period for livestock production (Deregibus et al. 1995; Jacobo et al. 2000). However, other perennial cool-season (*Leersia hexandra*, *Polypogon elongatus*, *Festuca arundinacea*, *Trifolium repens*) and warm-season (*Paspalum dilatatum*, *Bothriochloa laguroides*, *Lotus tenuis*) grasses and legumes species are also of high nutritional value and are preferred by cattle (Lemcoff et al. 1978; Cahuepe & Fernadez Grecco 1981; Cahuepe et al. 1985), but these species are dramatically affected by glyphosate application (Rodriguez & Jacobo 2010).

After disturbances that remove vegetation, the regeneration of the plant community depends on seed dispersal (Fenner & Thompson 2005) and on the availability of propagules in the soil, including seeds (Bakker & Berendse 1999; Bossuyt & Honnay 2008) and vegetative buds (Vesk & Westoby 2004). A severe disturbance, such as the application of a total systemic herbicide like glyphosate, may suppress the resprouting ability of the bud bank (Latzel et al. 2008). Therefore, the regeneration of above-ground vegetation would depend almost exclusively on the soil seed bank. In addition, this kind of disturbance may alter the size and composition of the seed bank because seed availability in the seed bank is closely related to seed production (Simpson et al. 1989). Therefore, disturbances that alter the composition of above-ground vegetation may change the relative contribution of seeds from each species.

The similarity of seed banks and above-ground vegetation among different types of grasslands generally is higher than other terrestrial ecosystems (Hopfensperger 2007). Among grasslands types, higher similarity occurred in frequently disturbed communities dominated by early-successional annuals compared to the perennial grasses of late-successional undisturbed grasslands (Moore 1980; Lavorel & Lebreton 1992). Supporting these findings, studies carried out to evaluate secondary succession trajectories in grassland systems reported lower similarity with successional time (Jensen 1998; Kalamees & Zobel 1998; Wagner et al. 2003).

Glyphosate application alters the vegetation composition of Flooding Pampa grassland (Rodriguez & Jacobo 2010) so that its use may alter the role of the seed bank in determining vegetation composition. Therefore, this study focused on two questions: (1) does the application of glyphosate affect the size, composition and diversity of the seed bank of Flooding Pampa grassland; and (2) to what extent does the seed bank determine the vegetation compositions of these grassland communities affected by this disturbance? The overall objective of this study was to determine if the seed bank remaining on glyphosate-treated sites has the potential to restore the original vegetation, by comparing the compositions of the soil seed bank of glyphosate-treated paddocks with the vegetation of untreated paddocks.

Methods

Study area

The Flooding Pampa is a 90 000-km^2 area in the eastern portion of the Pampa region (35° S–38° S, 58° W–61° W), where cow–calf operations are the main economic activity. The regional climate is temperate sub-humid, with mean annual precipitation varying from 1000 mm 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 the parent clay material, most soils belong to halo-hydromorphic complexes and associations influenced by flooding (Natraquolls, Natracualfs, Natralbolls and Argialbolls). The low fertility of soils and frequent flooding precludes the adoption of cultivated pastures or crops (Soriano et al. 1991).

The most widely distributed vegetation community is the humid mesophytic meadow, which develops along midland extended slopes (<3%). Along this slope, the upper or lower topographic position may determine differ-

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ences in the A_1 horizon depth, soil organic matter content (Batista et al. 2005), timing and duration of flooding events (Paruelo & Sala 1990) and species diversity, which may be organized as variants of the same community in either the upper and the lower position of the landscape (León 1975). 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). This community has been intensively exposed to glyphosate treatments during the last decade.

Sampling sites

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

Six paddocks dominated by the humid, mesophytic meadow community were selected from the farm rotational grazing system. Three of the six paddocks regularly received late-summer applications of glyphosate during the last 5 years and three paddocks were never herbicide-treated. A rate of 1440 g acid equivalent ha^{-1} of a commercial formulation of glyphosate (Roundup Full II®) with 100 $l{\cdot}ha^{-1}$ water was sprayed in a single application during the first week of March each year. Glyphosate was applied with a commercial terrestrial sprayer. As location along the topographic gradient may affect the response to glyphosate application, sites corresponding to the upper and lower position in each paddock were selected to measure the above-ground vegetation and seed bank.

Rainfall was recorded monthly during the study at the farm with a Hellman rain gage. The mean seasonal rainfall from 1999 to 2008 recorded at the farm was 345 mm in summer, 155 mm in autumn, 173 mm in winter and 287 mm in spring. Differences higher than ±25% compared to the average rainfall occurred during autumn and winter 2008, reaching values 41–69% lower than the mean.

Seed bank sampling and identification procedure

To collect the seed bank, 30 soil cores of 6.5-cm diameter and 7.0-cm depth were extracted with a drill from each site in the upper and lower position of each paddock. Subsamples were collected at least 2 m from each other, tracing a 'W' that covered the whole surface of each site. Total volume of soil extracted from each sample site was 1365 cm^3 (30 subsamples × 6.5-cm diameter × 7.0-cm depth), which exceed the minimum volume recommended by Hayashi & Numata (1971) and Roberts (1981). This grassland community is composed of both cool- and warm-season species, which shed their seeds in late spring and late summer and germinate in autumn and spring, respectively. In order to detect any seasonal differences in the composition of the seed bank, samples were taken during two seasons: late spring (December), after seed fall of cool-season species, and late autumn (June), after the seed fall of warm-season species. To assess two consecutive seasons, soil samples were extracted in December 2007 and June 2008 (first year) and in December 2008 and June 2009 (second year). The size, depth, number, horizontal distribution and timing of seed bank sampling followed the methodological considerations recommended by Csonos (2007).

Seedling emergence was used to estimate seed bank density and composition (Roberts 1981). Immediately after each extraction, subsamples were sieved to remove vegetation and litter. Soil with seeds recovered was spread in plastic trays (20 × 20 cm) on top of a layer (15 cm) of sterile soil, watered and put into a growth chamber (95 × 120 × 80 cm. BS 890, Bioamerican Science, Argentina) for 5 days. Temperature regime in the growth chamber was 25–35 °C for late-autumn samples and 10–20 °C for late-spring samples. Afterwards, trays were moved to a greenhouse with controlled temperatures and irrigated daily. Seedling emergence was recorded periodically. Emerged seedlings were allowed to grow until they could be identified, and subsequently removed to prevent seed contamination. About 5 months after seed bank sampling, the seed bank assay was completed, and the soil in each tray was sieved to determine if any seeds remained un-germinated. Seeds recovered in this way were placed in Petri dishes on cotton saturated with distilled water and put into a growth chamber to assess germination. The viability of un-germinated seeds was tested using tetrazolium blue (ISTA 1985). Nomenclature and naming authorities follow Flora del Cono Sur Database, from Instituto de Botánica Darwinion (2011).

Seed bank density and diversity

Seed bank density and each species seed density for the first (December 2007–June 2008) and second (December 2008–June 2009) year were calculated as the sum of seeds germinating from soil samples within each year, and expressed as the number of seeds germinating m^{-2}. As total soil area extracted from each site was 840 cm^2, the conversion factor was 11.9 to place on a m^{-2} basis. Seed bank composition in each year was assessed as the relative proportion of each seed species (number of seeds germinating m^{-2} $species^{-1}$ divided by the total number of seeds germinating m^{-2}). When there was no difference between years, seed bank data were combined to perform statistical analysis.

Species diversity of the seed bank was characterized for both richness and dominance. Species richness (*S*) was estimated as the total number of species found in the seed bank of each paddock. Dominance was estimated with 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 in each soil seed bank: $d = N_{\max}/N$, where $N_{\max}$ is the number of seeds of the most abundant species and N is the total number of seeds. This index ranges between 1 and 0, and the value increases as the soil seed bank 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 soil seed bank, because it provides a good estimate of diversity at relatively small sample sizes and is easily interpretable. The values range between 1 and 0, and the value increases as the soil seed bank becomes more even and rich (Magurran 2004).

Vegetation survey

To explore whether the composition of the soil seed bank was related to the above-ground vegetation, we recorded the basal cover of each plant species in the same sites from which seed banks were extracted in October 2008–February 2009 and October 2009–February 2010, following the methodology applied by Rodriguez & Jacobo (2010). Plant basal cover and species composition were estimated by the step-point method (Mueller-Dombois & Ellenberg 1974) along five to ten transects, which were each 10 m in length (200 points per transect) and randomly placed in along the upper and lower positions of each paddock.

We also performed vegetation sampling within each season, considering that almost all cool-season species occur in spring (October) and almost all warm-season species occur in summer (February). We recorded the seed bank and above-ground vegetation in the same season, and these were compared to each other. The seed bank recorded in the first year (December 2007–June 2008) was related to vegetation recorded in October 2008–February 2009, and the seed bank recorded in the second year (December 2008–June 2009) was related to vegetation recorded in October 2009–February 2010.

Statistical analysis

To evaluate differences in seed bank density, richness, dominance and diversity index, we performed two-way repeated-measures ANOVA, with position and glyphosate treatment as main factors and year as within-subject factor, because data of each year came from the same experimental units. For seed bank density analysis, the number of seed germinating m^{-2} was log-transformed to meet the homogeneity assumption of ANOVA. Contrasts of interest based on ANOVA results were conducted when interactions where significant. When the within-subject factor and its interactions were not significant, we averaged data from both years.

To describe the major sources of variation in seed bank composition between treatments and positions, ordination and classification multivariate techniques were performed. We carried out correspondence analysis (CA, Greenacre 1984) using frequency data (seeds germinating m^{-2} of each species divided by total seeds germinating m^{-2} in each sample unit). CA preserves chi-square distance (ter Braak 1985), which is the appropriate distance measure for variables expressed as samples weighted by their totals. CA may be applied to dimensionally homogeneous data tables, if the physical dimensions of the variables are the same (Perelman et al. 2003), which was true of our data matrix. To avoid giving too much emphasis to species whose abundance in the data matrix was low, we excluded species with constancy lower than 5%. We also performed a multi-response permutation procedure (MRPP) to test multivariate differences between glyphosate treatments and between positions, whereas season was averaged. We used relative Euclidean distance, weighting of groups *n*/sum (*n*) and rank transformed distance matrix. To identify particular species responsible for differences between these two groups, indicator species analysis and Monte Carlo tests (Dufrene & Legendre 1997) were performed using 1000 runs.

To analyse the relationship between above-ground vegetation and seed bank composition, we calculated the Sørensen similarity index (Looman & Campbell 1960) using Bray–Curtis ordination with two axes, Sørensen distance measure and Euclidean projection geometry. The Sørensen distance measures obtained for each treatment, position and year represented the dissimilarity Sørensen indices. Therefore, we used the reciprocal of these measures to obtain the similarity Sørensen index to perform two-way ANOVA with position and glyphosate treatment as main factors. Because the within-subject factor and its interactions were not significant, we averaged data in both years.

Univariate analysis two-way ANOVA and two-way repeated-measures ANOVA were performed using Statistica 1999 Edition (Statsoft Inc., Tulsa, OK, USA). Multivariate analysis RA (Decorana; Ecology and Systematics, Cornell University, Ithaca, NY, USA), MRPP (Mielke 1984) and indicator species analysis (Dufrene & Legendre 1997) were performed using PC-ORD TM version 4 (MjM Software Design, Gleneden Beach, OR, USA).

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Results

Seed bank density

Seed bank density varied greatly along the topographic gradient ($F = 1675.9$; $P < 0.001$) but was not affected by glyphosate treatment ($F = 0.5$; $P = 0.507$) or year ($F = 1.85$; $P = 0.209$). Seed bank density was seven times higher in the upper (42 400 ± 4121 seeds m^{-2}) than in the lower position (5760 ± 961 seeds m^{-2}).

Seed bank composition

The ordination of the seed bank surveyed in the upper and in the lower position showed that the principal gradient of variation in seed bank composition was glyphosate treatment. The first two axes of correspondence analysis accounted for almost 61% of total variance. The first axis reflected a shift in the seed bank composition between glyphosate-treated and untreated paddocks, as well as for the upper and the lower position (Fig. 1). The MRPP procedure confirmed that untreated paddocks belonged to one group (average distance among members = 0.308) and glyphosate-treated paddocks belonged to another group (average year), and that the composition of the seeds banks of these two groups were significantly different ($T = -14.04$, $P < 0.001$).

Species in the seed bank responsible for differences in the composition between glyphosate-treated and untreated paddocks were identified through indicator species analysis and Monte Carlo tests with a level of significance $P \leq 0.01$. Both in the upper and the lower position, a much higher proportion of seeds germinating of *Bromus catharticus*, *Digitaria sanguinalis* and *L. multiflorum* were recorded in glyphosate-treated than in untreated paddocks. In contrast, a much lower proportion of seeds germinated of *Cyperus rotundus*, *Dichondra microcalys*, *Lotus tenuis*, *Paspalum dilatatum* and *Stellaria media* were recorded in glyphosate-treated than in untreated paddocks. Seeds of *Bothriochloa laguroides*, *Eleocharis montevidensis*, *Juncus imbricatus*, *Leersia hexandra*, *Nassella neesiana* and *Festuca arundinacea* were only found in untreated paddocks (Appendix 1).

Seed bank diversity

Diversity was reduced by glyphosate application ($F = 53.4$, $P < 0.01$) in the upper position during both years (1 *df* contrasts; $P = 0.023$; $P < 0.001$ 2007–2008 and 2008–2009 periods, respectively) and in the lower position during the first year (December 2007–June 2008; 1 *df* contrast; $P < 0.001$). Therefore, differences in diversity index between years only occurred in the lower position when glyphosate was applied (1 *df* contrast; $P < 0.01$; Fig. 2).

Seed bank richness was severely reduced by glyphosate application ($F = 180.75$; $P < 0.001$), depending on topographic position (interaction treatment × position $F = 10.38$, $P < 0.05$) but not on season ($F = 3.02$, $P > 0.10$). The number of species in untreated paddocks was 21.17 ± 3.31 in the upper and 24.17 ± 3.43 in the lower position, while in glyphosate-treated paddocks richness was 13.50 ± 1.38 in the upper and 11.67 ± 1.67 in the lower position.

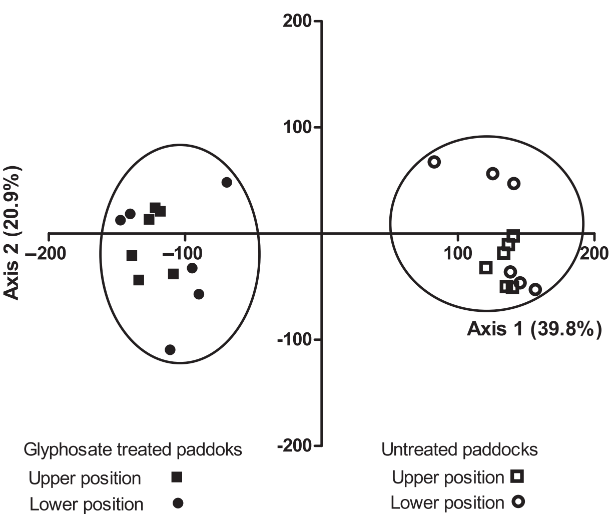

Fig. 1. Ordination (Correspondence Analysis) of seed bank composition corresponding to untreated and glyphosate treated paddocks of the upper and lower position. Ovals show the untreated group and the glyphosate treated paddocks group derived from MRPP procedure.

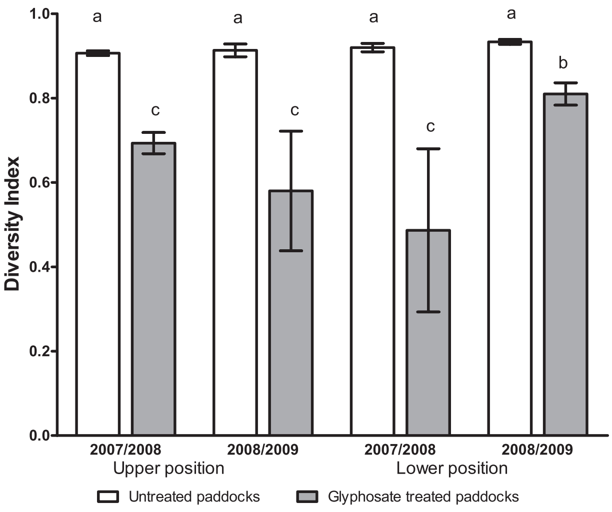

Fig. 2. Diversity index of seed banks in the upper and the lower slope position occurring in paddocks untreated (empty bars) and treated with late summer applications of glyphosate (grey bars) during the 2007/2008 and 2008/2009 years. Vertical lines indicate standard error of the mean. Significant differences are indicated with letters.

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Dominance was increased by glyphosate application (F = 180.72; P < 0.001). In untreated paddocks, dominance was 0.15 ± 0.02 on average, without any difference between positions (1 *df* contrast, P > 0.86) or year (1 *df* contrast; P > 0.80). In glyphosate-treated paddocks, dominance in the upper position was 0.57 ± 0.11 on average, without any difference between years (1 *df* contrast; P > 0.10). In the lower position, dominance was higher during the first than during the second year (0.69 ± 0.16 and 0.31 ± 0.05, respectively, 1 *df* contrast; P < 0.01).

Therefore, assemblages of the seed bank from glyphosate-treated paddocks were less rich and even, dominated by one species, mainly *L. multiflorum*, and consequently less diverse than assemblages from untreated paddocks.

Similarity between seed bank and above-ground vegetation composition

To assess the relationships between the compositions of the seed bank and the above-ground vegetation, the Sørensen similarity index was calculated. In the upper position, similarity among seed bank and vegetation composition was higher in glyphosate-treated than in untreated paddocks, while in the lower position, similarity did not differ among glyphosate treatments. The number of species recorded both in the seed bank and in the above-ground vegetation was lower in glyphosate-treated than in untreated paddocks, but the basal cover of the above-ground vegetation did not depend on either treatment or position (Table 1). Therefore, glyphosate application increased the similarity between seed bank and above-ground vegetation composition because only a few species (4–5) contributed more than 60% of above-ground cover in the upper position.

To predict to what extent the seed bank of the glyphosate-treated paddock would allow recovery of the original vegetation, we calculated the Sørensen similarity index between seed bank composition of glyphosate-treated paddocks and vegetation composition of untreated paddocks in the upper and in the lower position. Predicted similarity index in the upper position was 0.12 ± 0.03 and in the lower position was 0.16 ± 0.03. These predicted similarities were very low and reached lower values compared to similarity indices calculated for untreated paddocks in both upper (0.30 ± 0.05) and lower (0.29 ± 0.05) positions (contrasts; P < 0.05).

Discussion

The changes in seed bank produced by glyphosate application may have important implications for biodiversity conservation, vegetation recovery and livestock management in grasslands of the Pampas. Our results indicate that recurring late-summer applications of glyphosate produce a significant shift in seed bank composition, with reduced seed bank richness, diversity and increased dominance. These changes in plant community structure were also found in the humid mesophytic meadows of the Flooding Pampa (Rodriguez & Jacobo 2010).

Annual grasses and certain other species greatly increase in seed density with glyphosate treatment in these grasslands. Cool-season annual graminoids such as *L. multiflorum* and *B. catharticus* may escape elimination by glyphosate treatment because during the summer season these species are not actively growing and persist as seeds in the seed bank. Moreover, the timing of glyphosate application promotes seed germination and seedling growth of these species during autumn and, consequently, enhances their relative contribution to the plant community. In particular, *L. multiflorum* produces a larger quantity of seeds with a very high germination rate (Rodríguez et al. 1998), which are shed in late spring. The warm-season annual

Table 1. Two-way analysis of variance and descriptive statistics of Sørensen similarity index between seed bank and aboveground floristic composition, number of species and aboveground basal cover (%) of species recorded both in the seed bank and in the aboveground vegetation, in the upper and lower position from untreated and glyphosate treated paddocks. Values are the combined means of 2007/2008 and 2008/2009 years as within-subject effect and its interactions were not significant. Standard error of means are shown within parentheses. F ratio, degree of freedom (*df*) and *P* values resulting of the two-way analysis of variance for each variable are indicated (P: position; G: Glyphosate treatments).

Variable	Upper Position		Lower Position		Source of variance	*F*-ratio	*df*	*P*-value
	Untreated	Glyphosate	Untreated	Glyphosate				
Sørensen similarity index	0.3 (0.05)	0.5 (0.09)	0.3 (0.05)	0.3 (0.17)	P	9.86	1	0.013
					G	11.28	1	<0.01
					P × G	8.08	1	0.021
Number of species	11.2 (2.32)	4.5 (1.05)	15.7 (3.56)	5.2 (2.13)	P	7.28	1	0.027
					G	80.37	1	<0.01
					P × G	4.01	1	0.080
Basal cover (%) of species	53.7 (11.05)	63.0 (7.48)	58.1 (7.67)	47.7 (15.41)	P	0.02	1	0.89
					G	1.11	1	0.32
					P × G	0.98	1	0.35

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grass, *D. sanguinalis*, sheds seeds before glyphosate application, so that this species becomes an invasive weed in both in grasslands and in resistant (GR) soybean fields treated with glyphosate (Vitta et al. 2004; Scursoni & Satorre 2010).

In contrast, the seed bank densities of several perennial tussock grasses, sedges, dicots and legumes decreased dramatically when glyphosate was applied at the end of the summer. These species may be more susceptible to treatment then because the translocation rate of this systemic herbicide may be higher during times of rapid maturation and growth (Tworkoski & Sterrett 1987). For species whose main regeneration strategy is vegetative reproduction via tillering from basal axillaries buds, such as the perennial tussock grasses *L. hexandra*, *S. arundinaceus* and *P. dilatatum,* or by resprouting of below-ground storage and buds organs, such as the sedges *C. rotundus*, and *E. montevidensis*, recurrent glyphosate application may indirectly affect seed production by severely reducing their capacity for vegetative regeneration (Latzel et al. 2008; Webster et al. 2008; Reddy & Bryson 2009). For species whose main regeneration strategy is sexual reproduction, such as the warm-season perennial legume *L. tenuis* (Montes 1988) and annual, biannual or short-lived European herbs *L. taraxacoides*, *C. melitensis* and *P. lanceolata* (Marzocca et al. 1986), glyphosate application may affect seed production and seed viability via the interruption of both seed formation and seedling development (Darwent et al. 1994; Bennett & Shaw 2000; Clay & Griffin 2000; Baskin & Baskin 2001; Blackburn & Boutin 2003).

The reduction of seed bank richness in glyphosate-treated paddocks is related to the local extinction of several seed species: 16 species in the upper position (four native perennial grasses, two naturalized perennial grasses, three native perennial sedges and six naturalized dicotyledonous) and 12 species in the lower position (five perennial native grasses, three native perennial sedges and four dicotyledonous exotic annual–biennial species). The dramatic ten-fold increment of *L. multiflorum* seed density and the reduction of almost all other seed species increased dominance in the community. Higher dominance and lower richness threaten diversity conservation of plant communities (Fisher et al. 2009). For this reason, frequent application of glyphosate may be considered a severe disturbance responsible for seed richness and diversity losses, on a par with overgrazing, deforestation, fire or tillage (Freemark & Boutin 1995; Kalamees & Zobel 1998; Feng-Rui et al. 2009; Kassahun et al. 2009).

Inter-annual differences in diversity and dominance were found in the seed banks of the lower position between the 2007/2008 vs the 2008/2009 seasons. This difference may be related to drought in the autumn and spring of 2008, which may have reduced the tillering, flowering and seed production of *L. multiflorum.* This effect was evident only in the lower position, probably because of the shallower topsoil, lower soil organic matter content and clay texture (Batista et al. 2005), so that the water deficit may have been more severe in the lower position.

In our study, glyphosate application did not affect total density of seeds because the reduction in seeds quantities of functional groups damaged by herbicide application was counterbalanced by the increase in the pioneer species *L. multiflorum* (Omacini et al. 1995). Therefore, in glyphosate-treated paddocks, *L. multiflorum* was the main component of the density of the seed bank. Seed bank density was affected by position, according to the quality of each site. The lower position sustains a ten-fold lower seed bank because the soil is shallower and poorer than in the upper position (Batista et al. 2005). These environmental conditions are more restrictive for the growth of most species and, therefore, limit the capacity of these species to produce seeds and to replenish the seed bank. Several studies conducted in different ecosystems have also found a positive relation between environment and seed density (Osem et al. 2006; Feng-Rui et al. 2009; Kassahun et al. 2009).

To analyse the extent that seed bank determines vegetation composition of grassland communities affected by glyphosate, we calculated similarity between the seed bank and vegetation composition. The higher similarity index found in glyphosate-treated compared to untreated paddocks (50 and 30%, respectively) supports the idea that similarity between seed bank and vegetation is often higher in frequently disturbed early-successional than in less disturbed late-successional grasslands (Thompson & Grime 1979; Kalamees & Zobel 1998; Wagner et al. 2003). The higher similarity of seed bank and standing vegetation of disturbed paddocks is related to the increased dominance of *L. multiflorum*. The establishment of this species depends on water availability during each autumn, which is highly variable in the Flooding Pampa region. Consequently, grassland dominated by a single annual species exhibits low community stability.

Dramatic shifts in vegetation composition due to invasions of non-native annual grasses have been documented in various semi-arid and arid ecosystems originally dominated by native perennial species (Dyer & Rice 1999; Hamilton et al. 1999; Crall et al. 2006; DeFalco et al. 2007; Davies 2011; McGlone et al. 2012). Invasions of non-native species are facilitated by disturbances such as grazing, burning or tilling (Crawley 1987; Hobbs 1989; Hobbs & Huenneke 1992; Burke & Grime 1996). In some ecosystems, the shift in community composition from vegetation dominated by perennial native to exotic annual species requires widespread disturbance or stress (Corbin &

D'Antonio 2004). Consistent with these ideas, our results suggest that the seed bank of untreated paddocks may be a repository of species that mainly appear after disturbance, and the high dominance *L. multiflorum* is sustained by the recurrent application of glyphosate in temperate grassland of the Flooding Pampa.

To predict whether the seed bank remaining on glyphosate-treated sites has potential to restore the vegetation composition, we related the soil seed bank of glyphosate-treated paddocks with the vegetation of untreated paddocks, obtaining a very low similarity index (17%). For restoration purposes, the main role of the seed bank is to restore the target dominant species, typical of undisturbed communities (Honnay et al. 2002). After glyphosate treatment, the target species (native perennial grasses) are absent or rare in the seed bank, so that the seed bank would play a minor role in vegetation recovery. Therefore, the restoration of grassland species composition would depend mainly on propagule dispersal from adjacent areas, mainly via cattle, wild mammals, birds, insects or wind (Fenner & Thompson 2005). The dispersal potential of the target species often depends on the dispersal ability of each species, the distance from the viable source of target species to the restoration site, and the presence of dispersal vectors at a landscape scale (Donath et al. 2003). If propagules did not arrive, secondary succession after glyphosate spraying ended would drive the community composition to a quite different assemblage compared to the original vegetation assemblage.

Conclusions

Our study demonstrated that recurrent glyphosate application changes species composition, floristic richness and diversity of the seed bank and the above-ground vegetation of the humid mesophytic meadows of the Flooding Pampa grassland. As these seed bank changes involve the local extinction of native perennials and other species, community restoration would depend mainly on propagules dispersing from adjacent areas. Our results raise awareness about the risks of widespread herbicide application for biodiversity conservation in the last semi-natural habitats in the Pampas grasslands.

Our results also have implications for livestock production in grasslands of the Flooding Pampa. The critical period for cattle production occurs during winter, when forage growth is very low. Livestock managers frequently apply glyphosate in late summer. This practice increases *L. multiflorum* production, which provides high-quality forage and is less expensive than feeding livestock with cultivated cool-season forage species. However, when glyphosate is applied, winter forage production depends exclusively on a single annual species, *L. multiflorum*, so that germination and establishment are determined solely by the climate conditions of each autumn. When water is available, the practice of glyphosate application increases forage production in winter. However, rainfall in March is highly variable and water availability is not always adequate for *L. multiflorum* germination so that forage yield may be very variable using this approach. Therefore, glyphosate application may pose a great risk to livestock managers.

Other ecologically and economically sustainable practices exist to improve winter forage production in this region, such as applying rotational grazing methodology that promotes the early establishment of *L. multiflorum* Lam. (Jacobo et al. 2000), or rotational grazing with phosphate fertilization, which increases the production of C_3 annual grasses and legumes (Rodríguez et al. 2007). Both alternatives reduce the seasonal variability of plant production by increasing winter forage production and lead to an improvement in rangeland condition and carrying capacity (from 0.6 to 1.0 Animal Units ha^{-1}). These alternative practices increase the proportion of perennial high forage value species, reduce the cover of low forage value species and maintain a high floristic diversity (Jacobo et al. 2006). These alternative methods also support the same level of forage productivity as that obtained by applying herbicide, but these practices do not compromise the ecological sustainability of the grassland.

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Appendix 1

Average number of seeds germinating m^{-2} of species registered in seed banks of untreated and glyphosate treated paddocks from the upper and lower slope positions.

Treatment	Upper slope position		Lower slope position		*P*-value	Lc	O
	Untreated	Glyphosate	Untreated	Glyphosate			
Cool season annual grasses							
Bromus catharticus Vahl	161 (127)	2168 (1085)	172 (105)	248 (110)	<0.01	AB	N
Lolium multiflorum Lam.	1750 (609)	24053 (4510)	1375 (91)	2563 (1334)	<0.01	A	E
Poa annua L.	123 (154)	225 (322)	27 (24)	38 (53)	0.68	A	E
Cool season perennial grasses							
Leersia hexandra Sw.	658 (658)		117 (259)		<0.01	P	N
Nassella neesiana (Trin. & Rupr.) Barkworth	72 (32)		13 (8)		0.01	P	N
Polypogon elongatus Kunth	66 (113)	37 (45)	16 (18)	22 (43)	0.75	P	N
Festuca arundinacea Schreb.	49 (62)		160 (274)		<0.01	P	E
Warm season annual grasses							
Digitaria sanguinalis (L.) Scop.	34 (61)	1626 (306)	302 (10)	598 (873)	<0.01	A	N

Appendix 1 (Continued)

Treatment	Upper slope position		Lower slope position		*P*-value	Lc	O
	Untreated	Glyphosate	Untreated	Glyphosate			
Warm season perennial tussock grasses							
Agrostis stolonifera L.	153 (96)		30 (88)		0.06	P	E
Bothriochloa laguroides (DC.) Herter	481 (234)		36 (98)		0.01	P	N
Distichlis spicata (L.) Greene	8 (20)		12 (36)		1.00	P	N
Paspalum dilatatum Poir	4659 (313)	87 (192)	184 (364)	10 (22)	<0.01	P	N
Setaria parviflora (Poir.) Kerguélen	1749 (798)	1006 (1492)	184 (107)	231 (218)	0.12	P	N
Sporobolus indicus (L.) R. Br.	1614 (1421)	670 (736)	149 (235)	95 (105)	0.19	P	N
Sedges							
Carex sp.	2371 (629)				1.00	P	N
Cyperus rotundus L.	6460 (1165)	383 (310)	541 (102)	26 (48)	<0.01	P	N
Eleocharis montevidensis Kunth	2371 (827)		161 (47)		0.01	P	N
Juncus imbricatus Laharpe	35 (39)		89 (145)		<0.01	P	E
Juncus sp.	18 (45)		6 (13)		1.00	P	N
Sisyrinchium platense I.M. Johnst.	502 (398)	79 (194)	116 (90)		0.47	P	N
Legumes							
Lotus tenuis Mill.	5197 (943)	392 (298)	318 (292)	80 (102)	<0.01	P	N
Medicago lupulina L.	292 (714)	224 (348)	50 (113)	48 (43)	1.00	P	E
Dicotyledonous herbs							
Ammi visnaga (L.) Lam.		271 (149)			0.47	AB	E
Anthemis cotula L.		112 (79)			1.00	A	E
Apium sellowianum H. Wolff.	9 (22)	201 (297)	2 (5)	2 (5)	0.16	AB	E
Capsella bursa-pastoris (L.) Medik.	97 (71)		81 (113)	17 (30)	0.46	AB	E
Carduus acanthoides L.	28 (46)	159 (246)	10 (37)		0.56	A	E
Carduus nutans L.				6 (14)	1.00	B	E
Centaurea melitensis L.	784 (247)		89 (85)	23 (56)	<0.01	AB	E
Centaurium pulchellum (Sw.) Druce	46 (44)		4 (14)		0.47	A	E
Cichorium intybus L.		81 (75)			1.00	AB	E
Cirsium vulgare (Savi) Ten.		195 (146)			0.44	AB	E
Dichondra microcalys (Hallier f.) Fabris	161 (170)	18 (28)	159 (164)	82 (68)	<0.01	P	N
Fumaria officinalis L.		198 (146)			0.47	A	E
Gamochaeta americana (Mill.) Wedd.		317 (400)	27 (71)	7 (18)	1.00	BP	N
Leontodon taraxacoides (Vill.) Mérat	1275 (592)		182 (99)	11 (28)	<0.01	AB	E
Mentha pulegium L.	946 (519)	1727 (854)	138 (62)	228 (301)	0.67	P	E
Oxalis articulata Savigny	92 (150)	6 (15)	39 (37)	20 (31)	0.18	P	N
Phyla canescens (Kunth) Greene	151 (150)		22 (21)	45 (103)	0.18	P	N
Plantago lanceolata L.	2009 (1620)		277 (215)	91 (70)	<0.01	P	E
Plantago major L.	668 (628)	509 (608)	86 (42)	93 (64)	0.48	P	E
Polygonum aviculare L.	229 (168)	9 (19)	33 (20)		0.18	AB	E
Portulaca oleracea L.	9 (18)	9 (19)	3 (5)	3 (4)	1.00	A	E
Rumex crispus L.				5 (10)	1.00	P	E
Stellaria media (L.) Cirillo	298 (188)	317 (492)	77 (51)	27 (21)	<0.01	A	E
Taraxacum officinale G. Wever ex F.H. Wigg.	22 (22)		5 (5)		0.46	P	E
Veronica polita Fr.	228 (168)	9 (18)	33 (20)	18 (37)	0.18	A	E

Data are the mean of 2007–2008 and 2008–2009 years and standard deviations are shown within parenthesis. *P* values resulting of Monte Carlo test of significance of Indicator Species Analysis are shown. Lc: Life cycle (A: annual, B: biannual, P: perennial) and O: origin (N: native, E: exotic) are indicated.

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