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Glyphosate Alters Aboveground Net Primary Production, Soil Organic Carbon, and Nutrients in Pampean Grasslands (Argentina)☆

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ABSTRACT

We have previously demonstrated that recurrent application of glyphosate causes dramatic shift in the vegetation structure of the native grasslands of Flooding Pampa. As these structural changes might alter functional processes such as primary production, carbon, nitrogen and phosphorus cycling, this study aims to evaluate functional changes associated with the application of glyphosate in these temperate grasslands. We measured aboveground net primary production (ANPP) during two consecutive years, and the concentration of organic carbon, nitrogen and phosphorus in the soil during the following six years after primary production measurements ended in glyphosate treated and non- treated (control) paddocks of a commercial livestock far. We related the vegetation data, basal cover, species richness and diversity, obtained in a previous study conducted in the same paddocks of the livestock farm, with ANPP data obtained in this one. Late summer applications of glyphosate greatly reduced the biomass contribution of warm-season perennial grasses and legumes and increased the contribution of cool season annual grasses, altering the seasonal pattern of ANPP. As the reduction of the spring and summer productivity could not be compensated by the increase of cool-season productivity, the annual ANPP was lower in the glyphosate-treated paddocks than in control paddocks. Glyphosate applications also decreased soil organic carbon and phosphorus concentration, probably because of the reduction of ANPP, the changes of its seasonal distribution and the shift in the floristic composition of the community, which may modify the amount and quality of the litter. We found a linear positive relationship between basal cover, species richness and species diversity with ANPP, which suggest that the negative effects on ecosystem functioning would be directly related with the changes in vegetation structure caused by glyphosate application.

Introduction

Grasslands are exposed to anthropogenic disturbances that may alter their structural and functional traits (Gelbard, 2003). Disturbances, such as grazing of domestic herbivores and fire, affect the interaction among grassland components, which may drive changes in vegetation community traits, such as floristic and seed bank composition and diversity (Tilman, 1982). In turn, changes in vegetation community traits may alter ecosystem-level processes such as aboveground net primary productivity. Richness, diversity, or identity of species or functional groups have great influence over several ecosystem processes (Hooper et al., 2005). Numerous experiments have found that plant species diversity is positively related to plant biomass (Tilman et al., 1996, 2001, 2012; Hector et al., 1999; Cardinale et al., 2011), the abundance of animals (Borer et al., 2012), and soil organic carbon storage (Fornara and Tilman, 2008; Steinbeiss et al., 2008), while richness is positively related to the water content in the soil surface horizon (Lange et al., 2014) and the abundance of decomposers (Eisenhauer et al., 2010, 2011). All these variables are strongly associated with the carbon and water cycle (Allan et al., 2013). The identity of species or functional groups determines litter quality and, therefore, the rate of litter decomposition (Bardgett and Shine, 1999), which regulates the content of organic carbon and nitrogen in soils (Sharif et al., 1994). Functional group composition, particularly the presence of legumes, has strong effects on ecosystem processes associated with the nitrogen cycle (Allan et al., 2013). Disturbances that cause changes in vegetation community structure might affect aboveground and belowground plant productivity, the quantity and quality of litter, and soil biotic community structure and function, driving changes in ecosystem processes such as carbon and nutrient cycles (Hobbs and Huenneke, 1996).

The application of herbicides is considered as a main disturbance that causes a biodiversity reduction in temperate agroecosystems (Freemark and Boutin, 1995). Broad-spectrum herbicides, such as glyphosate, alter biodiversity and ecosystem functioning (Helander et al., 2012). They cause shifts in weed population by increasing annual broad-leaved species (Johnson et al., 2009), reduce plant species richness and diversity, and affect seedling growth and biomass production of nontarget plants

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(Tesfamariam et al., 2009). Glyphosate also alters soil microbial communities (Druille et al., 2015) that drive carbon and nutrient cycling.

In the Flooding Pampa grasslands (Argentina), the spraying of glyphosate has become an extended practice. Glyphosate is applied in late summer to eliminate vegetation composed mainly of C_4 grasses and forbs to improve germination and establishment of cool-season (C_3) annual grasses, whose major component is *Lolium multiflorum* Lam. However, recurrent application of glyphosate dramatically alters the vegetation community structure. It greatly reduces species richness and diversity, causes the local extinction of several native perennial species, increases the basal cover of exotic cool-season annual grasses, and reduces the basal cover of cool-season perennial grasses, warm-season tussock grasses, warm-season legumes, and the vegetation cover in summer (Rodríguez and Jacobo, 2010). Therefore, glyphosate-induced structural vegetation changes may alter functional processes, such as primary production, carbon, nitrogen, and phosphorus cycling in the Flooding Pampa grasslands.

The aims of this study are 1) to evaluate whether glyphosate application alters aboveground net primary productivity (ANPP), organic carbon (SOC), nitrogen (N), and phosphorus (P) concentration in soils and 2) to explore the association between functional and structural vegetation changes of the Flooding Pampa grasslands. We carried out a farm-scale experiment to test that recurrent application of glyphosate alters the seasonal patterns of ANPP and reduces organic carbon, nitrogen, and phosphorus concentration in soil. We also tested whether ANPP changes are related to the changes in vegetation structure variables, such as total basal cover, richness, and diversity.

Methods

Study Area

The Flooding Pampa is a 90 000-km^2 area in the eastern portion of the Pampa region where cow-calf operations to produce yearlings is the main economic activity. Because of its flat relief, the occurrence of a high water table, and the nature of parent material, most soils of the area belong to the halo-hydromorphic complexes and associations influenced by flooding (Natraquols, Natracualfs, Natralbols, and Argialbols). Well-drained soils (Hapludols and Argiudols) 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 (Perelman et al., 2001). The regional climate is temperate, subhumid, with mean annual precipitation varying from 1 000 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. The favorable climate condition allows forage production throughout the year because of the sequential growth of C_3 and C_4 grasses during the cool and warm seasons, respectively. However, forage production is lower in winter, which restricts the carrying capacity of this system (Deregibus et al., 1995). In the past 15 years, the application of glyphosate has been promoted to increase winter forage productivity in the native grassland of the Flooding Pampa. Glyphosate is sprayed in late summer to eliminate green vegetation composed by C_4 grasses and forbs in order to improve germination and establishment of cool-season (C_3) annual grasses, whose major component is *Lolium multiflorum* Lam., a naturalized species widespread in the region.

We conducted the study on a commercial 1 600-ha farm located in Azul (Buenos Aires Province), in the central area of the Flooding Pampa (36°40′S, 59°32′W, 80 m asl.). The main activity of the farm is Angus and Hereford cow-calf operations. Rotational grazing is performed on 60-ha grassland paddocks with an average stocking rate of 0.8 AU ha^{-1}. The occupation period in each paddock varies between 3 and 15 days, and the rest period is between 25 and 90 days, according to the growth rate of major forage species. The most extended plant community of the paddocks was the "humid mesophytic meadows," dominated by *Lolium multifl*orum Lam., *Paspalum dilatatum* Poir., *Bothriochloa laguroides* (DC.) Herter, *Sporobolus indicus* (L.) R. Br., *Panicum milioides* Nees ex Trin., *Nassella neesiana* (Trin. & Rupr.) Barkworth., *Chascolytrum subaristatum* Lam., *Piptochaetium montevidensis* (Spreng.) Parodi, and *Danthonia montevidensis* Hack. & Arechav. (Perelman et al., 2001). The dominant soil of the paddocks is a typical Natraquoll, chararacterized by an acidic, nonsaline A_1 horizon 12-cm depth and a clayey and sodic B_{2t} horizon. The very hard and compact B_{2t} horizon limits water and air movements and root elongation, so root biomass and soil biological activity is concentrated in the upper horizon (Lavado and Taboada, 1988). We have previously conducted other studies on this farm to evaluate the effect of recurrent application of glyphosate on vegetation (Rodríguez and Jacobo, 2010) and seed bank composition (Rodríguez and Jacobo, 2013).

Experimental Design

We selected the same six 60-ha paddocks studied in previous research work (Rodríguez and Jacobo, 2010, 2013). Botanical composition of these paddocks was registered before randomly assigning the treatments to ensure that the dominant grassland vegetation was the "humid mesophytic meadows" community. Three paddocks regularly received late-summer application of glyphosate during 5 yr, whereas the other three paddocks were not treated (control). Glyphosate was applied with terrestrial spray equipment at a dose of four to five l ha^{-1} in a single application during the first week in March each year. At this time, warm-season species are growing actively and perennial cool-season species start their growing period.

Plant biomass samples were obtained during two consecutive experimental periods: April 2006 – March 2007 and April 2007 – March 2008. Soil samples were extracted in March 2008, 2010, and 2012. Vegetation structural variables—total basal cover, richness, and diversity—were provided by a previous experiment performed at the same experimental units (Rodríguez and Jacobo, 2010, see Supplementary material, available online at http://dx.doi.org/10.1016/j.rama.2017.07.009).

Rainfall was recorded monthly on the farm during both experimental periods. During the first experimental period, annual rainfall was 7% higher and during the second experimental period was 16% lower than the historical 1979 – 2008 average (961.6 mm), respectively.

Aboveground Net Primary Production

To estimate aboveground net primary production (ANPP), in December 2006, three 1-m^2 cages were randomly located in each paddock to exclude cattle grazing. Biomass was hand-harvested at ground level with scissors. To avoid biomass loss due to senescence, harvest frequency was adjusted to the growth rate of the vegetation, resulting in six harvests during each experimental period. Biomass samples were separated into eight components: standing dead material and seven functional groups, 1) cool-season (C_3) annual grasses, 2) cool-season (C_3) perennial grasses, 3) warm-season (C_4) tussock grasses; 4) warm-season (C_4) creeping grasses; 5) legumes, 6) dicotyledonous forbs, and 7) sedges. Biomass components were oven-dried to constant weight at 70°C, and then they were weighed. Growth rates were calculated as the biomass increment between two consecutive harvests, and these data were processed on a monthly basis. Therefore, a monthly ANPP pattern from April 2006 to March 2008 was obtained. The annual ANPP was calculated as the addition of monthly values for the first (April 2006 – March 2007) and the second experimental periods (April 2007 – March 2008). For each experimental period, the contribution of each biomass component or functional group to the winter-spring ANPP (July – December) and the summer-autumn ANPP (January – June) was calculated.

Vegetation Structure Variables

Total basal cover, richness, and diversity data were obtained in a previous experiment performed in the same paddocks (Rodríguez and

Jacobo, 2010). In that study, plant basal cover and species composition were estimated using the step-point method (Mueller-Dombois and Ellenberg, 1974) along five 10-m-long transects (200 points per transect), randomly placed in each paddock. Vegetation was surveyed six times: spring (October), summer (February), and late summer (April) of two consecutive experimental periods 2006 − 2007 and 2007 − 2008. Bare soil, litter, or standing dead material was recorded where no living plants were intercepted. Species richness was estimated as the total number of species per paddock, and species diversity was determined calculating the Gini-Simpson's diversity index, $1\text{-D} = 1\text{-}\sum \mathrm{pi}^2$, where p is the proportion of each species in each paddock (see Supplementary material, available online at http://dx.doi.org/10.1016/j.rama.2017.07.009).

Total basal cover of living plants recorded during spring and summer was correlated with the ANPP registered in spring (October-November-December) and summer (January-February-March) of the first 2006 − 2007 and second 2007 − 2008 experimental periods, respectively. Richness and diversity indices estimated for each experimental period were correlated with annual ANPP recorded in the corresponding experimental period.

Organic Carbon, Phosphorus, and Nitrogen Concentration in Soil

Soil organic carbon (SOC), total nitrogen (N), and total phosphorus (P) concentration were determined by extracting 10 soil subsamples per paddock at 12-cm depth. As we expected that soil traits would change in a longer time scale and would show a lower intra-annual variation (Chaneton et al., 1996) than changes in vegetation structure and functioning, soil subsamples were extracted only in April 2008, 2010, and 2012. Subsamples were air-dried, 2 mm sieved, and composed in one composite sample per paddock. SOC was determined by Walkley and Black (1934) method, N content was measured by Kjeldahl method, and P content was determined by perchloric acid digestion (Page et al., 1982).

Statistical Analysis

To evaluate differences in ANPP and the contribution of functional groups to the winter-spring and summer-autumn ANPP, repeated-measures analysis of variance were performed, considering glyphosate treatments as main effect and experimental periods as within-subject effect. To evaluate the effect of glyphosate application on ANPP patterns, double-repeated-measures analysis of variance were performed, considering glyphosate treatments as a main effect and experimental periods and months as double within-subject effects. Contrasts of interest based on ANOVA results were conducted when interactions were significant using the Tukey test. The relationships among structural and functional variables were tested using regression models. The effects of glyphosate application on soil organic carbon (SOC), total nitrogen (N), and total phosphorus (P) concentration were evaluated using repeated-measures analysis of variance, considering glyphosate treatments as a main effect and years as within-subject effect, after being arc-sin transformed. Statistica software (StatSoft, Inc., Palo Alto, CA) was used to perform statistical analyses.

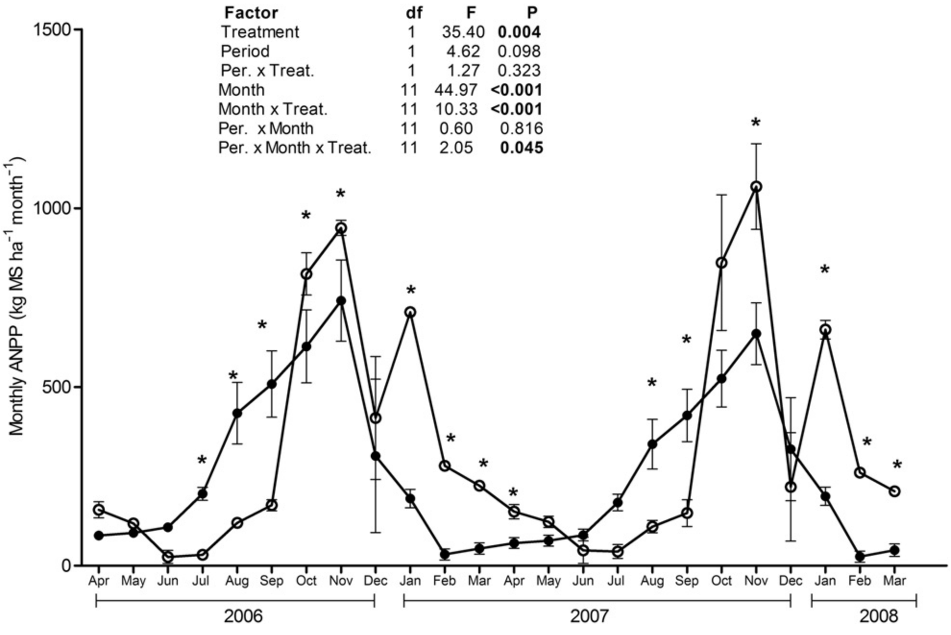
Results

Aboveground Net Primary Production and Contribution of Functional Groups

Glyphosate application affected the pattern of ANPP during both experimental periods. ANPP in glyphosate-treated paddocks was higher during winter (July − September) and lower during spring (October − November) and summer (January − March) than in the control paddocks (Fig. 1).

Annual ANPP in glyphosate-treated paddocks was lower than in the control paddocks both in the first April 2006 − March 2007 period (3 350 ± 190 vs. 4 005 ± 97 kg DM ha^{-1} period^{-1}) and in the second April 2007 − March 2008 experimental period (2 918 ± 207 vs. 3 871 ± 354 kg DM ha^{-1} period^{-1}) (Treat: F = 35.4, $P = 0.004$, Period: F = 4.6, $P = 0.09$, Treat. × Period: F = 1.2, $P = 0.32$).

Relative and absolute contribution of functional groups to the ANPP during the winter-spring and the summer-autumn seasons were affected by glyphosate applications and were similar between experimental periods. During the winter-spring season, relative and absolute contributions of cool-season annual grasses, whose major component was *Lolium multiflorum*, were higher, while the contribution of cool-season perennial grasses was lower in glyphosate-treated paddocks than in control paddocks. During the summer-autumn season, the relative and absolute contributions of warm-season creeping grasses, dicotyledonous forbs,



Factor	df	F	P
Treatment	1	35.40	**0.004**
Period	1	4.62	0.098
Per. x Treat.	1	1.27	0.323
Month	11	44.97	**<0.001**
Month x Treat.	11	10.33	**<0.001**
Per. x Month	11	0.60	0.816
Per. x Month x Treat.	11	2.05	**0.045**

Figure 1. Monthly pattern of aboveground net primary production in control *(open symbols)* and glyphosate-treated paddocks *(black symbols)* from April 2006 to March 2008. *Vertical bars* show 1SE of the means. *Asterisks* indicate significant differences among treatments within each month ($P < 0.05$). Insert: degree of freedom *(df)* F and P statistics for the main factor (Treatment) and within-subject effects (Periods and Month) and their interactions that resulted from the double-repeated measures analysis of variance.

Table 1

A, Relative and **B**, absolute contributions of functional groups to the aboveground net primary production during the winter-spring and summer-autumn seasons in control and glyphosate-treated paddocks

Functional groups	Winter-spring						Summer-autumn					
	Control		glyphosate		*F*	*P*	Control		glyphosate		*F*	*P*
	Mean	SE	Mean	SE			Mean	SE	Mean	SE		
A. Relative contribution (%)												
Cool-season annual grasses	5.03	5.23	85.66	6.80	265.01	< 0.001	9.36	1.16	4.96	2.50	7.63	0.051
Cool-season perennial grasses	28.28	2.32	0.62	0.74	386.01	< 0.001	19.58	10.42	0.36	0.09	10.21	0.033
Warm-season tussock grasses	0.51	0.61	0.01	0.00	2.01	0.229	32.61	3.09	6.84	5.00	57.70	0.002
Warm-season creeping grasses	0.85	0.85	0.01	0.01	2.93	0.162	5.16	3.68	21.04	0.43	55.11	0.002
Legumes	20.88	12.45	0.21	0.17	5.00	0.111	8.10	2.63	1.50	1.50	14.27	0.019
Sedges	3.56	3.72	0.29	0.48	2.28	0.205	7.67	4.71	0.66	0.66	6.52	0.063
Dicots forbs	28.18	19.14	0.15	0.25	6.43	0.064	0.26	0.09	18.65	0.65	23.58	< 0.001
Standing dead material	12.61	3.27	13.07	7.92	0.01	0.930	17.26	0.52	46.00	6.97	50.81	0.002
B. Absolute contribution (kg DM·ha^{-1}·period^{-1})												
Cool-season annual Grasses	124	43	2248	104	119.00	< 0.001	137	2	26	4	162.523	< 0.001
cool-season Perennial grasses	695	19	16	6	400.72	< 0.001	281	45	2	0	13.071	0.022
Warm-season tussock Grasses	13	5	0	0	2.01	0.229	484	29	35	9	74.915	0.001
Warm-season creeping Grasses	21	7	0	0	2.94	0.161	79	21	109	1	0.694	0.452
Legumes	514	102	5	1	8.22	0.046	121	15	8	3	18.406	0.013
Sedges	88	31	7	4	2.26	0.208	116	24	3	1	7.028	0.057
Dicots forbs	692	156	4	2	6.49	0.063	4	0	96	2	1029.683	< 0.001
Standing dead material	310	27	336	62	0.05	0.836	256	10	238	12	0.454	0.538

and standing dead material were higher while the contribution of warm season tussock grasses was almost five times lower in glyphosate-treated paddocks than in control paddocks (Table 1, A and B).

Relationship Among Structural Variables and ANPP

A positive linear regression was found among total basal cover and ANPP recorded in spring and summer in glyphosate-treated and control paddocks ($R^2 = 0.86$, F = 131.4, $P < 0.001$). During summer 2007 and 2008, the basal cover of glyphosate-treated paddocks was lower than 40% and PPNA was 265 kg DM.ha^{-1} season^{-1} on average, while in control paddocks basal cover ranged 55 − 70% and ANPP reached 1160 kg DM.ha^{-1} period^{-1} on average. During spring 2006 and 2007, glyphosate-treated and control paddocks basal cover ranged between 78% and 98% and ANPP was 1 800 kg DM.ha^{-1} season^{-1} on average (Fig. 2A). Glyphosate-treated and control paddocks were discriminated along the species richness and diversity regression lines (Fig. 2B and C). The relation between richness and ANPP ($R^2 = 0.75$, F = 29.6, $P < 0.001$) showed that when the number of species ranged between 15 and 20, annual ANPP ranged between 2 700 and 3 500 kg DM.ha^{-1} period^{-1}, while when the number of species was higher than 50, annual ANPP reached values from 3 700 to 4 300 kg DM.ha^{-1} period^{-1}, corresponding to glyphosate-treated and control paddocks, respectively (Fig. 2B). Species diversity and PPNA also showed a strong positive linear relation ($R^2 = 0.77$, F = 33.8, $P < 0.001$) presenting diversity index values lower than 0.82 (registered in glyphosate-treated paddocks) related to ANPP values between 2 700 and 3 500 kg DM.ha^{-1} period^{-1}, and diversity index values higher than 0.89 (registered in control paddocks), which corresponded to ANPP values between 3 700 and 4 300 kg DM.ha^{-1} period^{-1} (Fig. 2C).

Organic Carbon, Phosphorus, and Nitrogen Content in Soil

Glyphosate applications reduced organic carbon and phosphorus content in soil (Fig. 3A and B) but did not significantly affect nitrogen content (Fig. 3C). Organic carbon content gradually decreased in glyphosate-treated paddocks, and in 2012 it was 12% lower than in control paddocks (F = 36.7, $P = 0.003$) (see Fig. 3A). Average P content during the whole period was lower in glyphosate-treated paddocks than in control paddocks (5.4 vs. 6.1 ppm, respectively) (see Fig. 3B). Although N content did not show significant differences among treatments and interaction (Fig. 3C), the contrast between glyphosate treatments in 2012 showed a trend to be lower in control paddocks (F1 = 6.5, $P = 0.08$).

Discussion

We found that late-summer applications of glyphosate reduced the annual amount of primary production and changed its seasonal pattern, decreased organic carbon and phosphorus content in soils, and tended to reduce soil nitrogen content of the Flooding Pampa grasslands. The reduction of ANPP in spring and summer in glyphosate-treated paddocks resulted from the decrease of the relative and absolute biomass contribution of warm-season functional groups. This response was consistent with the drastic decrease of basal cover to < 20% recorded in summer because of the significant decline of warm-season tussock grasses and legumes basal cover (Rodríguez and Jacobo, 2010). Particularly, warm-season tussock grasses provide the bulk of forage in summer (Deregibus et al., 1995). Its main components, *P. dilatatum* and *B. laguroides,* are tall native grasses of high-forage quality (Jacobo et al., 2006). In contrast, during winter ANPP was higher in glyphosate-treated paddocks due to the significant increase of the relative and absolute biomass contribution of cool-season annual grasses, whose main component, *L. multiflorum* (Rodríguez and Jacobo, 2010), exhibits much higher growth rates in winter than other cool-season native or exotic perennial grasses (Lemaire and Agnusdei, 2000). The drastic reduction of absolute and relative biomass of warm-season (C_4) tussock grasses, which mainly contribute to the generation of leaf area in summer, was not counterbalanced by the increase of cool-season (C_3) annual grasses so as to maintain similar annual primary production in glyphosate-treated and control paddocks. For this reason, ANPP in glyphosate-treated paddocks was lower than in control ones. Arzadun and Mestelan (2009) reported similar results when comparing the effect of late-summer glyphosate application in small experimental plots (8 m^2) in the Flooding Pampa grassland. They found that the greater dry matter yield of *L. multiflorum* did not compensate for the decrease in *Lotus tenuis* and other warm-season grasses, resulting in a decrease of almost 30% of total annual yield. This response may be partially explained by the differences between C_3 and C_4 photosynthetic metabolisms, each one having a set of traits that, when coexisting, confer ecological benefits to occupy diverse habitats and climatic seasonal variations (Johnston, 1996). It is widely known that in summer, with high temperatures and higher light intensity and atmospheric demand, C_4 grasses use water more efficiently than C_3 grasses because C_4

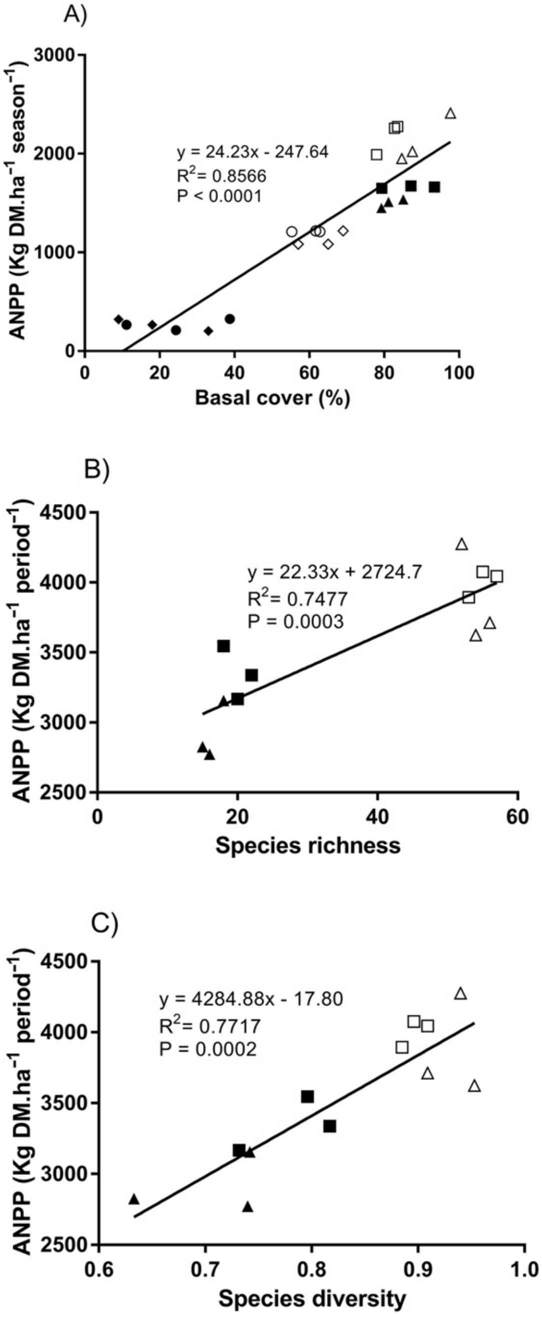


Figure 2. Regressions between **A,** plant basal cover, **B,** richness, and **C,** diversity with aboveground net primary production (ANPP) registered in glyphosate-treated paddocks *(black symbols)* and in control paddocks *(open symbols)*. **A,** Plant basal cover and ANPP registered during spring 2006 *(squares)*, spring 2007 *(triangles)*, summer 2007 *(circles)*, and summer 2008 *(rhombs)*. **B,** Richness and ANPP registered in April 2006 — March 2007 *(squares)* and April 2007 — March 2008 *(triangles)* experimental period. **C,** Diversity and ANPP registered in April 2006 — March 2007 *(squares)* and April 2007 — March 2008 *(triangles)* experimental period. Insert: equation model, R^2 and P statistic resulted from the linear regression analysis.

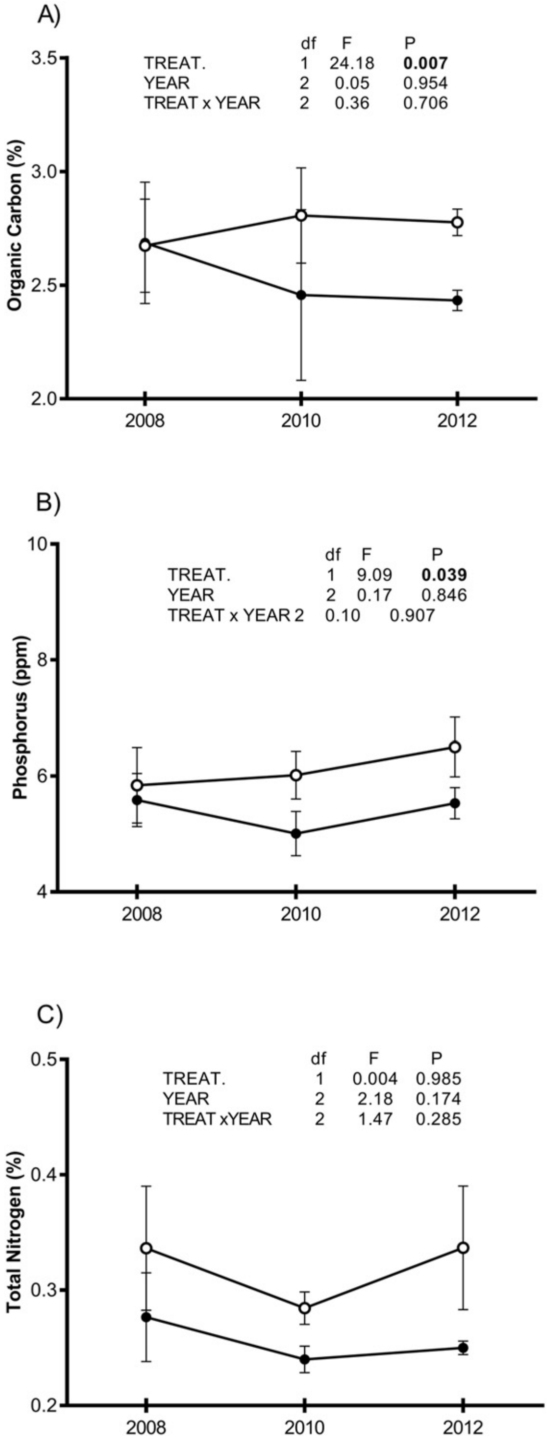


Figure 3. Pattern of organic carbon **A,** total phosphorus **B,** and total nitrogen **C,** in soils in control *(open symbols)* and glyphosate-treated paddocks *(black symbols)* from 2008 to 2012. *Vertical bars* show 1SE of the means. Insert: degree of freedom *(df)* *F* and *P* statistics for the main factor (Treatment) and within-subject effect (YEAR) and their interactions resulted from the repeated measures analysis of variance.

metabolism operates with low stomatal conductance, allowing carbon fixation at lower water potential than that of C_3 (Raschke, 1975). In contrast, photorespiration in C_3 species increases with light intensity and temperatures > 25°C, reducing net carbon fixation (Osmond et al., 1982). In addition, the optimum temperature for photosynthesis in C_4 plants is generally higher than in C_3 ones (30 — 40°C vs. 10 — 25°C) and light saturation is reached at greater light intensity and leaf area index in C_4 than in C_3 plants (Long, 1999). The latter require maintaining open stomata and rapid gas exchange with the atmosphere at lower light intensities and temperatures to maximize photosynthetic rate (Osmond et al., 1982). Therefore, in 1-yr time-scale, the drastic reduction of C_4 tussock grasses cannot be compensated by the increase in C_3 annual grasses.

In experimental grassland communities, Marquard et al. (2009) demonstrated that the positive relationship between species richness and productivity was explained by the increase in community tiller density, induced by species diversity. They suggested that the increase in tiller density in richer and more diverse communities would result from a higher availability of sites for germination or establishment, as intraspecific neighbors were replaced by interspecific ones, reducing overlap in resource niches. We also found that changes in primary productivity mediated by glyphosate application were positively associated with basal cover, species richness, and diversity. Assuming that in grasslands, living plant basal cover is positively related with module density, the reduction of basal cover in glyphosate-treated paddocks during summer may result from the reduction of module or plant density in a less rich and diverse community, where high intraspecific competition maximizes overlap in the use of resources.

It is well known that the amount of the aboveground and belowground biomass and the abundance, identity, and diversity of plant species determine the quantity, quality, and diversity of litter, which in turn control the rate of litter decomposition and mineralization in soil organic matter, among other biotic and abiotic factors (Aerts et al., 2003). These are key processes in the cycles of carbon and nutrients in pastoral ecosystems (Bardgett and Shine, 1999). In turn, this reduction of detritus supply for microorganisms may determine a reduction in soil macro-organism and microorganism activity, responsible for organic matter humification and litter decomposition (Raich and Tufekcioglu, 2000). After 10 yr of recurrent glyphosate applications, organic carbon and phosphorus content in soil decreased while nitrogen content tended to be lower when compared with control paddocks. This response can be attributed to the changes of vegetation functioning and structure, which may modify litter amount and quality. Litter amount would be lower due to the reduction of the ANPP during spring and summer and, probably, belowground biomass. A reduction of root biomass in glyphosate-treated paddocks is expected due to the dominance of annual grasses species and the concomitant reduction of perennial grasses, as root biomass of perennial grasses used to be three times higher than that of annual grasses (Mapfumo et al., 2002). Litter quality would be reduced due to the higher contribution of standing dead material in summer and the lower contribution of legumes, thus increasing the C:N ratio and lignin content of plant tissues (Taylor et al., 1989). The soil microorganism activity may be depressed by the microclimate conditions during summer, as recurrent glyphosate applications increased bare soil to 80% (Rodríguez and Jacobo, 2010), causing lower moisture and higher temperature at soil level (Rodrigo et al., 1997). Soil microorganisms may also be affected by the toxic effect of glyphosate, as it was demonstrated for symbiotic ones and arbuscular mycorrhizal fungi (Zobiole et al., 2011; Druille et al., 2015). These factors would lead to a restriction in the quantity and quality of the substrate to decompose and in the activity of soil microorganism, explaining the reduction of organic carbon and soil P content (Knops et al., 2010).

It is widely accepted that many ecosystem processes depend on species or functional groups diversity (Hooper et al., 2005). In many ecosystems, plant species richness is positively correlated with plant biomass, decomposers abundance, carbon, and nutrient cycling rates (Allan et al., 2013). Plant litter has a critical role in these relationships, and it determines soil properties and substrate supply for microorganisms (Wardle and Lavelle, 1997). In temperate grasslands, increasing litter diversity increases the efficiency of soil biological processes, such as decomposition and nutrient turnover (Bardgett and Shine, 1999). Supported by this conceptual framework, our results suggest that recurrent application of glyphosate alter several bio-geo chemical ecosystem processes mediated by the reduction of plant richness, diversity, and productivity.

Implications

Application of glyphosate in the native grassland of the Flooding Pampa has been promoted to increase winter ANPP. This practice increases winter forage production per hectare but greatly reduces the summer and annual ANPP. In turn, these changes in vegetation functioning may explain the reduction of organic carbon and phosphorus content in soils. Therefore, the short-term benefit of obtaining higher winter-spring forage is overcome by the reduction of forage production in summer, the decrease of annual ANPP, and soil fertility in the long term. Our study also demonstrates that the recurrent spraying of glyphosate in the Flooding Pampa grassland negatively affects ecosystem functioning and warns about the risk for biodiversity loss in this seminatural habitat in the Pampas grasslands.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.rama.2017.07.009.

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