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Effects of High Impact Grazing on Species Diversity and Plant Functional Groups in Grasslands of Northern Argentina

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Abstract: High impact grazing (HIG) was proposed as a management option to reduce standing dead biomass in Northern Argentinean (Chaco) rangelands. However, the effects of HIG on grassland diversity and shifts in plant functional groups are largely unknown but essential to assess the sustainability of the impact. During a two-year grazing experiment, HIG was applied every month to analyze the seasonal effects on plant species composition and plant functional groups. The results indicate that irrespective of the season in which HIG was applied, the diversity parameters were not negatively affected. Species richness, the Shannon–Wiener diversity index and the Shannon’s equitability index did not differ from the control site within a 12-month period after HIG. While plant functional groups of dicotyledonous and annual species could not benefit from the HIG disturbance, C₃-, C₄-monocotyledonous and perennials increased their absolute and relative green cover. Our results suggest that HIG, if not applied in shorter frequencies than a year, neither alters diversity nor shifts the plant species composition of the grassland plant community, but instead it promotes previously established rather competitive species. HIG could therefore contribute as an alternative management practice to the sustainable land use intensification of the “Gran Chaco” grassland ecosystem and even counteract the encroachment of “low value” species.

Keywords: Chaco; Corrientes; biomass; management; rangeland

1. Introduction

Natural and semi-natural tropical grassland ecosystems comprise around 11% of the terrestrial land surface [1–3]. These ecosystems provide multifunctional services such as biomass for livestock grazing, carbon and water storage and the conservation of floral and faunal biodiversity [4]. In Northern Argentina, the Chaco phytogeographic region comprises large areas of combined semi-natural grasslands and forests [5], and is considered a diversity hot spot of Southern America [6,7].

Among all factors influencing the plant species diversity of natural or semi-natural grasslands, land management has the potential to exert the largest impact [8–10]. Therefore, sustainable land use practices mediating between goals of livestock production and grassland diversity conservation are of major concern [11,12].

In general, the effects of grazing on plant species composition and diversity are well documented for most of the world’s grassland ecosystems [13–17]. However, contrasting results regarding

reduced, unaffected or even increased diversity or shifts in plant functional groups were reported. These different responses to herbivores were mainly explained by environmental gradients of available resources such as nutrients, water or energy gradients, which are used to compensate for the loss of biomass due to grazing [10,18,19]. The anthropogenic impact via grazing management strongly intervenes in natural ecosystem processes such as nutrient cycles or the water balance [19] and affect the system's resilience. Most literature dealing with grazing intensity relates specifically to management with continuous grazing. The latter is determined by the stocking rates, compared to carrying capacity [16,20]. Nevertheless, sustainable grazing management must avoid overstocking and overgrazing [9,21]. Overstocking is avoided by ensuring that livestock do not exceed the amount of forage available to feed them and to leave sufficient biomass to ensure the ecosystem functions. Overgrazing is avoided by having short grazing periods, achieved by some form of rotational grazing [16,22,23]. In a sustainable rotation, the number of cattle and the grazing and resting periods are adjusted to each environment [16,24,25]. In a rotational grazing, stocking rates can be much higher than with continuous grazing and the grazing intensity will be low even at much higher stocking rates than for a continuously grazed pasture. Nevertheless, there is no evidence demonstrating that rotational grazing is superior in terms of enhanced plant and animal production compared to continuous grazing on rangelands [16,22]. The general goal of grazing management should seek to avoid grassland degradation by ensuring ecological functions and services. The anthropogenic impact via grazing management strongly interacts with natural ecosystem processes capturing solar energy in nutrient cycles and water balance [19] and affecting the system's resilience. Mismanagement or often so-called "overgrazing" can lead to strong grassland degradation, with ecological functions and services being temporarily or permanently reduced.

Due to the low year-round stocking rates in the grasslands of Northern Argentina, which are applied to adapt to the limited fodder availability during the winter, grasslands accumulate large amounts of plant material resulting from the vigorous growth of C₄ grasses during the summer growing season [26]. The surplus of standing biomass substantially decreases the amount of solar energy, as dead material shades out the sward, taller species dominate and reduce the presence of shorter species and thus biodiversity [27,28]. As compared to traditional methods to reduce standing dead biomass (SDB) such as burning, ploughing and mowing, high impact grazing (HIG) was analyzed as a successful alternative management option to reduce SDB [26]. This method is based on the natural destructive impact of large and dense herds of herbivores found in natural grasslands [26,29–31]. Although being successful in reducing SDB and even promoting the re-growth of the vegetation, the effects of HIG on plant diversity and the floristic composition of the grassland are still unclear.

It was found that areas excluded from grazing had lower species richness and diversity indexes compared to permanently grazed areas [32]. For humid areas of Central Argentina, it was found that on midslopes, rotational grazing increased the cover of C₃ grasses, while in lowlands, plant functional groups remained unaffected [25]. Specifically, in the Chaco region, it was found that species diversity and evenness decreased, while species richness remained unaffected after eight years with a continuously high stocking rate [16].

Analyses of plant–animal interactions in grassland ecosystems usually consider grazing disturbance as a permanent pressure or episodic impact by grazers, the latter usually practiced in rotational grazing systems. However, for short-term-pulse, high-impact grazing events we are still missing experimental evidence from field trials. Besides this, there are just few studies analyzing the effects of grazing intensity on species composition in sub-humid tropical grasslands. This study aims to analyze the effects of HIG on grassland floristic composition, diversity and plant functional groups. The results of this study will contribute to an improved understanding of HIG with regard to contra-productive or complimentary effects on the dual goals of grassland diversity conservation and sustainable intensification.

2. Materials and Methods

2.1. Study Area

The study was conducted on the Corrientes INTA (National Institute of Agriculture) Research Station (1175 ha) in the province of Corrientes situated in northeast Argentina, located in the Chaqueño Oriental phyto-geographic district [33], 30 km South (lat. 27°40'23.27" S, long. 58°44'12.94" W, 69 m.a.s.l.) from the Corrientes capital city. The annual mean temperature is 21.3 °C. The average temperature for the coldest month July is 15.6 °C and of the warmest month January is 27.1 °C. Local mean annual precipitation is ~1300 mm. Most of this occurs in autumn (33% from March to May) and summer (30% from December to February), and less in spring (24% from September to November) and winter (13% from June to August). Sandy-loam texture soils (Aquic Argiudol) dominate in the study area [34]. Soils remain moist or very moist for most of the year, due to the high precipitation and the clay layer at approximately 40 cm depth (Bt horizon). The pH varies between 5.6 and 6.0 and soil organic matter varies from 1.2 to 1.7% in the upper soil layer.

The grassland vegetation reaches 2 m in height with an annual net primary productivity of up to 15 t ha⁻¹ dry matter (DM), which is dominated by *Andropogon lateralis* Nees and *Sorghastrum nutans* (L.) Nash interspersed with small shrubs and trees [6]. Poaceae C₄ species are the most dominant plant functional group in the grasslands of northern Argentina, comprising bunch and short grasses with medium to moderate nutritional quality for ruminants [35]. Besides the productive C₄ grasses, mainly *Cyperaceae* species (sedges) with medium to low nutritional value and C₃ *Fabaceae* species (legumes) with a higher protein content [7], contribute to the total aboveground biomass. Forage growth is strongly seasonal, with the maximum standing green biomass during summer (December–February) and minimum during winter, between July–September [36]. Cattle graze freely at medium to relatively low stocking rates (~0.5 animal unit ha⁻¹) all year round [37].

2.2. Experimental Layout

The experiment was established on a 24 ha natural grassland area, which is part of the research facility of the INTA Corrientes, that was previously managed with continuous grazing at an intensity of 0.5 animal units per ha⁻¹ year⁻¹. The HIG treatment was applied in three replicates of 6 ha following a monthly sequence on adjacent sub-plots of 12 × 0.5 ha in each replicate. For that purpose, a mixed 75-animal herd of Braford, Hereford, and Brahman cattle breeds was used, representing an instantaneous grazing intensity of 150 animal units ha⁻¹. HIG was carried out in the e-fenced subplot (50 × 100 m) until vegetation was completely grazed and standing biomass trampled down and partially incorporated into the upper soil layer. HIG was compared to a control site with no HIG but continuous grazing with 0.5 animal units per ha⁻¹ year⁻¹. The experiment started in July 2012 and ran until June 2013; all 12 subplots of each replicate with HIG were treated in a different month. After the impact the subplot was continuously grazed at 0.5 animal units per ha⁻¹ year⁻¹, to resemble the average stocking rate of 0.5 animal unit ha⁻¹ in Corrientes Province [37].

2.3. Grassland Species Composition, Diversity and Plant Functional Groups

A detailed plant species inventory was performed at the biomass peak time in the summer during February, 2014. The least area size that sufficiently represented the species richness was defined to be 8 m² ($p \leq 0.05$). During this inventory and at five randomly chosen positions within each of the 36 HIG sub-plots, we visually estimated the total ground cover of the standing dead biomass (SDB) and the green biomass ground cover (GB) of each individual species as well as the share of litter and bare soil. For the control plot, sub-plots of similar size were analyzed at randomly selected positions. The sampled sub-plots represented the status of the grassland between 1 and 12 months after HIG. In total, 200 sub-plots of 8 m² size were analyzed. Additionally, to follow the seasonal dynamics, monthly ground cover rankings for individual species were made from July 2013 to July 2014 on 5 smaller size quadrats of 25 × 25 cm (20 each month).

This study defined and measured species richness (S) as the total number of plant species within the sampling plots. The Shannon–Wiener diversity index (H) was calculated considering S and the evenness of individual (plant) species [38,39]. The Shannon’s equitability (E) index was used to indicate how evenly different species are distributed. All plant species were also categorized to their botanical families and to their plant functional groups (PFGs): monocotyledons and dicotyledons, photosynthesis pathway (C_3 – C_4) and life cycle (perennial and annual).

2.4. Statistical Analysis

We analyzed the effects of HIG applied every month, compared to the control areas without treatment. The experiment was set up as a block design with three replicates (R1–R3). A linear mixed model for repeated measures using maximum likelihood (REML) in time with independent heteroscedastic errors was used to fit a serial and spatial variance covariance structure to compensate for autocorrelation using a spherical covariance structure [40]. All variables (GB, SDB, H, E, S and PFGs) were analyzed as dependent variables in the regression analysis. The comparison of means was tested when a significant F-value was achieved; then the least significant difference (LSD) post hoc analysis was applied. The significance level was set at $\alpha = 0.05$.

Principal component analysis (PCA) was used to ordinate plant functional groups and diversity to HIG impact. When working with vegetation data, the arch in many calculated PCA plots was suggested as a distortion indicating an environmental gradient. To avoid possible effects for PCA analysis, we selected a homogeneous experimental area with the same soil and vegetation properties at the beginning of the experiment. Samples were randomly collected and there was no zonation or gradient detected (lat. $27^{\circ}40'17.42''$ S, long. $58^{\circ}44'13.09''$ W). Additionally, we did not use PCA for the analysis of individual species, we rather analyzed the different variables in relation to HIG disturbance. PCA analysis indicated the maximum possible variability; as a result, it identifies the most important sources of variation. PCA analysis helps to explain the variability introduced on the measured variables, due to HIG timing, compared to the control. The orthogonality of the main components ensures that the PC2 provides new information on variability with respect to that provided by PC1, it explains the variability produced by HIG not explained by PC1. Mixed model analysis was performed with SAS (v. 9.3) and PCA with InfoStat (v.2014).

3. Results

3.1. Plant Species Identified on the Plots

In total, we identified 166 different plant species belonging to 37 families on the HIG sub-plots and on the control area (Table A1). Most species belonged to the *Poaceae* (62%), *Cyperaceae* (21%), and *Asteraceae* (3%) families. Besides that, 60% of all species were dicots and the rest monocots. Perennial species dominated (82%) over annuals (18%). C_4 species represented 54%, C_3 species 41% and crassulacean acid metabolism (CAM) species made up the rest (5%).

3.2. Standing Dead and Green Biomass Ground Cover

The standing dead biomass based on ground cover (%) was strongly reduced after HIG grazing (generally between 4–20%) compared to the control site with on average 65% SDB (Table 1). The green biomass at HIG plots increased within one year, corresponding to up to more than 80% compared to the control, with only around 30% GB.

Table 1. Mean values (\pm sd.) of green (SGB) and standing dead biomass (SDB) in % of ground cover. Mean values (\pm sd.) of plant functional groups: C₃, C₄ species, monocots, dicots, annual and perennial species based on % of green ground cover. Means (\pm sd.) for the diversity parameters plant species richness (S), diversity (H) and evenness (E). Measurements taken at the peak biomass time in February 2014 between 19 and 357 days after high impact grazing (HIG). Means with different letters are different with $p \leq 0.05$.

Impact Season	Impact Month	Days after HIG	SGB (%)		SDB (%)		C ₃ (%)		C ₄ (%)		Monocots (%)		Dicots (%)		Annual Species (%)		Perennial Species (%)		S		H		E	
	Control	-	25.0 \pm 8.3	d	66.7 \pm 13.0	a	12.3 \pm 4.6	ab	12.7 \pm 4.6	a	17.4 \pm 1.7	a	7.6 \pm 1.7	ns	1.1 \pm 1.0	ns	23.9 \pm 1.0	a	48.0 \pm 6.9	ns	2.6 \pm 0.4	ns	0.66 \pm 0.06	ns
Summer	February	19	18.8 \pm 5.9	d	45.1 \pm 4.6	b	8.0 \pm 5.4	a	10.7 \pm 5.5	a	17.9 \pm 0.6	a	0.9 \pm 0.6	ns	0.0005 \pm 0.001	ns	18.8 \pm 0.0009	a	42.3 \pm 5.4	ns	1.8 \pm 0.3	ns	0.58 \pm 0.07	ns
	January	52	67.4 \pm 7.8	ac	16.2 \pm 6.1	ce	23.3 \pm 5.9	bcd	43.6 \pm 6.6	bcd	60.7 \pm 4.6	bcd	6.7 \pm 4.6	ns	0.7 \pm 0.2	ns	66.7 \pm 0.2	d	42.0 \pm 5.4	ns	2.5 \pm 0.1	ns	0.67 \pm 0.02	ns
	December	54	62.8 \pm 9.7	bc	18.5 \pm 8.0	cd	32.1 \pm 18.5	d	30.6 \pm 18.6	b	45.8 \pm 18.7	b	17.0 \pm 18.7	ns	2.1 \pm 1.5	ns	60.7 \pm 1.5	bc	43.0 \pm 6.0	ns	2.4 \pm 0.1	ns	0.63 \pm 0.05	ns
Spring	November	87	67.8 \pm 6.5	ac	10.3 \pm 5.2	cf	16.8 \pm 8.6	abc	50.9 \pm 8.8	cd	57.1 \pm 9.1	bcd	10.7 \pm 9.1	ns	2.3 \pm 3.3	ns	65.5 \pm 3.3	cd	43.0 \pm 6.1	ns	2.6 \pm 0.2	ns	0.70 \pm 0.04	ns
	October	116	59.8 \pm 4.0	c	4.4 \pm 0.8	f	11.4 \pm 7.0	ab	48.4 \pm 7.0	cd	53.6 \pm 2.3	bc	6.2 \pm 2.3	ns	0.8 \pm 0.8	ns	59.0 \pm 0.8	b	36.3 \pm 5.3	ns	2.5 \pm 0.2	ns	0.69 \pm 0.03	ns
	September	146	71.6 \pm 16.1	ac	8.4 \pm 3.4	def	21.6 \pm 3.9	abcd	49.8 \pm 3.9	cd	64.6 \pm 2.3	cd	7.0 \pm 2.3	ns	1.0 \pm 1.2	ns	70.6 \pm 1.2	de	36.3 \pm 5.3	ns	2.4 \pm 0.1	ns	0.66 \pm 0.08	ns
Winter	August	176	78.2 \pm 9.6	a	11.2 \pm 5.9	def	31.7 \pm 15.0	cd	46.1 \pm 14.4	cd	57.9 \pm 20.3	bcd	20.3 \pm 20.3	ns	1.7 \pm 2.1	ns	76.5 \pm 2.1	fg	40.7 \pm 7.2	ns	2.5 \pm 0.2	ns	0.66 \pm 0.06	ns
	July	206	67.8 \pm 4.0	ac	20.1 \pm 7.3	c	27.5 \pm 3.4	cd	40.2 \pm 3.4	bc	56.9 \pm 10.4	cd	10.9 \pm 10.4	ns	2.3 \pm 2.7	ns	65.5 \pm 2.7	cd	44.3 \pm 7.2	ns	2.5 \pm 0.3	ns	0.67 \pm 0.06	ns
	June	253	78.3 \pm 1.5	a	5.1 \pm 1.5	ef	20.5 \pm 7.5	abcd	57.8 \pm 7.4	d	64.6 \pm 4.4	cd	13.7 \pm 4.4	ns	5.9 \pm 7.2	ns	72.4 \pm 7.2	ef	51.7 \pm 7.7	ns	2.9 \pm 0.1	ns	0.73 \pm 0.01	ns
Autumn	May	285	77.6 \pm 2.9	ab	7.0 \pm 1.2	def	22.9 \pm 6.5	abcd	54.6 \pm 6.5	cd	62.1 \pm 12.9	bcd	15.5 \pm 12.9	ns	2.5 \pm 3.4	ns	75.1 \pm 3.4	efg	45.7 \pm 7.8	ns	2.9 \pm 0.3	ns	0.75 \pm 0.03	ns
	April	317	77.9 \pm 4.1	a	7.8 \pm 3.3	def	26.7 \pm 10.6	cd	51.0 \pm 10.8	cd	62.5 \pm 13.9	bcd	15.4 \pm 13.9	ns	4.0 \pm 7.0	ns	73.9 \pm 7.0	efg	44.0 \pm 7.7	ns	2.7 \pm 0.3	ns	0.71 \pm 0.03	ns
	March	357	80.4 \pm 3.2	a	7.2 \pm 0.4	def	25.7 \pm 10.3	cd	54.5 \pm 10.3	cd	73.4 \pm 4.7	d	7.0 \pm 4.7	ns	1.5 \pm 1.6	ns	78.9 \pm 1.6	g	50.0 \pm 6.5	ns	2.7 \pm 0.2	ns	0.69 \pm 0.05	ns

3.3. Green Biomass Ground Cover of Plant Functional Groups

The relative shares of monocotyledonous, C₃ and C₄ plant species and perennial plant species were significantly affected by HIG (Table 1). The C₄ species green cover strongly increased to an average of 45% on HIG sub-plots (ranging from 10.7 to 57.8%) while for the control it was significantly lower, with 12.7% (sd. = 4.6). The C₃ species increased after HIG from 8.0% (sd. = 5.4) to max. 32.1% (sd. = 18.5) cover, while in the control they averaged 12.3% (sd. = 4.6). HIG applied in winter and autumn at least doubled the cover of C₃ species compared to the control sub-plots, while when applied in summer and spring it produced a similar C₃ cover to the control. The monocotyledonous plant species green cover strongly increased by around 200% after HIG compared to the control (Table 1). No effect was found for dicot ground cover. Similarly, perennial species cover strongly increased after HIG compared to the control, but HIG did not affect the annual species cover, which represented less than 6% throughout all analyzed plots.

If attributed to plant functional groups, most of the species (close to 80%) showed a positive response to HIG by increasing their green biomass share (Table 2). We could not identify a functional group among monocotyledonous, dicotyledonous, annual, perennial, C₃, C₄ and CAM species that decreased with regard to green biomass cover after HIG. A total of 80% of monocotyledonous species (mostly *Poaceae*) increased and only 3% decreased. Nevertheless, dicotyledonous species were not negatively impacted by HIG, but showed a lesser increase (67%) and greater decrease (17%) compared to monocotyledonous species (Tables 2 and A1).

Table 2. Plant functional groups with indifferent, decreasing or increasing relative green biomass cover in response to HIG (high impact grazing). For the full data set view Table A1 in Appendix A.

Plant Functional Groups	Indifferent		Decrease		Increase	
	Number of Species	%	Number of Species	%	Number of Species	%
Dicotyledonous	16	17	16	17	64	67
Monocotyledonous	11	17	2	3	52	80
Perennials	24	18	13	10	95	72
Annuals	3	10	6	19	22	71
C ₃	10	15	10	15	48	71
C ₄	16	18	9	10	64	72
CAM	1	13		-	7	88

3.4. Diversity

Species richness (S) measured at the peak biomass time in February was not affected by HIG and its seasonal timing. While at the control site we recorded on average 48 (sd. = 6.9) species in our 8 m² sample units, the HIG sites had between 36 and 52 species (Table 1). Also, the Shannon–Wiener diversity index (H) and Shannon’s equitability index (E) did not reveal any difference between the HIG and control sites.

3.5. Relative Green Biomass Share of Selected Plant Families and Species

HIG affected the relative biomass cover shares of the *Poaceae*, *Cyperaceae*, *Fabaceae*, and *Asteraceae* families, together accounting for more than 99% of the green biomass ground cover (Figure 1). The relative green cover shares of *Poaceae* were between 59 and 65%, with no difference between the HIG and control sites. In contrast, relative green biomass cover of the *Cyperaceae* species strongly increased after HIG, from 4% to 18–27%. On the other hand, the relative green cover of *Fabaceae* was not reduced significantly after HIG (4.4% control, 2–2.6% HIG). The relative green biomass cover of the *Asteraceae* species was 10.3% in the control sub-plots, and was strongly reduced after HIG to 0.8–1.1%. The seasonal impact timing of HIG had no effect on the shares of these main plant families.

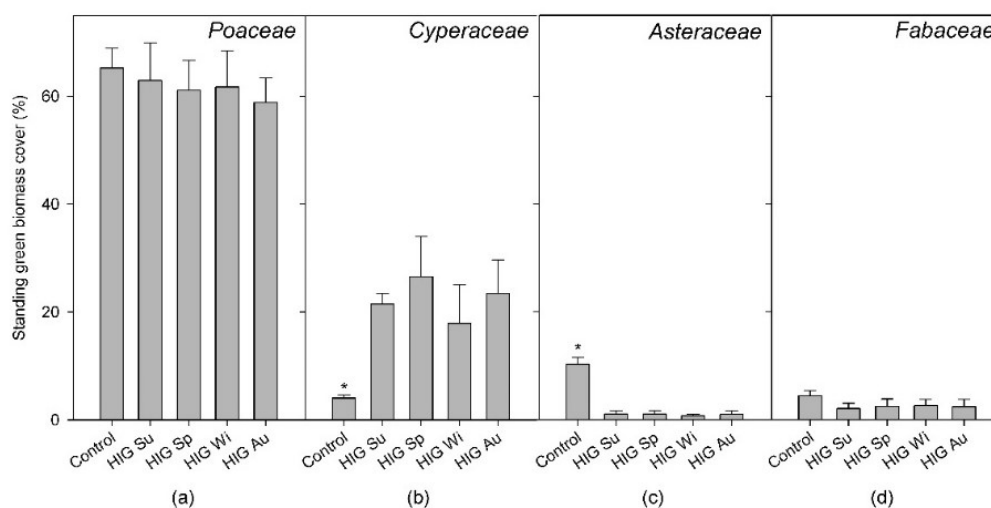


Figure 1. Effect of high impact grazing (HIG) and its seasonal timing on the relative shares of green biomass cover for the botanical families of (a) *Poaceae*, (b) *Cyperaceae*, (c) *Asteraceae* and (d) *Fabaceae* sampled at the HIG and control sites during the peak biomass time (February 2014). (HIG Su = HIG applied in Summer; HIG Sp = HIG applied in Spring; HIG Wi = HIG applied in Winter and HIG Au, HIG applied in Autumn. Error bars indicate the standard error (se) of the means ($p \leq 0.05$).

3.6. Principal Component Analysis (PCA)

The two PCA axes can explain 90% of the total variation in the data set (Figure 2). The plant functional groups of dicots and monocots, C_4 and C_3 species as well as perennials and annuals showed an antagonistic relationship. Green cover and dead material are also naturally antagonistic. The diversity parameters species richness (S), Shannon index (H), and evenness (E) are much more related to the appearance of annuals and dicots rather than perennials and monocots. Summer, autumn and winter HIG were more closely related to the appearance of perennials and monocots, while HIG in spring was more closely related to annuals and dicots. The spring HIG was more closely related to the diversity parameters E and H compared to all other treatments. The control was mostly related to dicots, dead material and C_3 species, however also S and annual species were positively correlated.

According to our interpretation, the PC1 opposes the variable control with all HIG variables. HIG in autumn and in winter are not particularly associated with any classification criteria. PCA also revealed that HIG applied in summer, autumn or winter produced monocots and perennials, as opposed to the control, which was characterized by dicots, C_3 and deterrent dead material. Besides this, the PC2 found that HIG in autumn is associated with bare ground (due to the intense trampling), which, as we already demonstrated in a previous study, reduces the grassland's productivity [26]. Applied in autumn, HIG may be the least sustainable option. Finally, the PC2 also showed that HIG in the spring promoted not only green cover and C_4 , but also diversity, as it is associated with E, H and annuals.

3.7. Monthly Monitoring of Diversity One Year after HIG

To analyze the seasonal dynamics of diversity as affected by HIG, we sampled species composition every month for one entire year on plots where HIG was applied exactly one year ago. Data were compared to samplings taken at the same time from control plots. H, E and S for HIG and control sub-plots showed a similar increasing general trend (Figure 3) and were not significantly different between HIG and the control plots. The regression analysis indicated that the slopes for both HIG and the control plots were not different ($p < 0.05$).

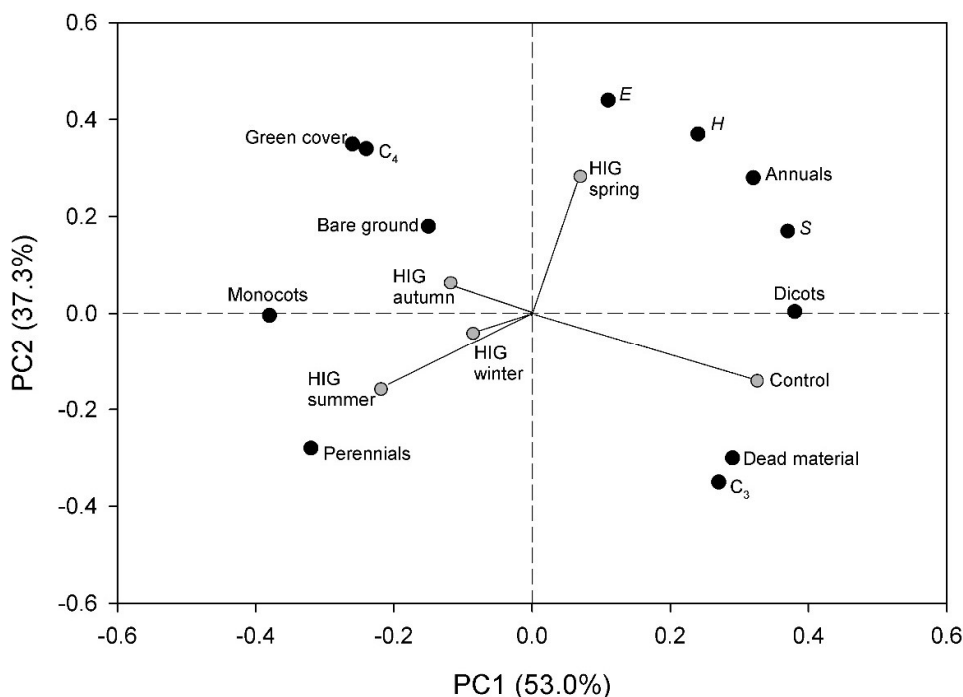


Figure 2. Biplot of the principal component analysis (PCA), both axis together explain 90% of the data set variation (PC1 53% and PC2 37%). The classification criteria were species richness (S), species diversity (H) and species evenness (E), green and standing dead biomass cover, ground cover of plant functional groups: annuals, perennials, C₃, C₄, dicots and monocots. High impact grazing (HIG) applied in winter, spring, summer or autumn.

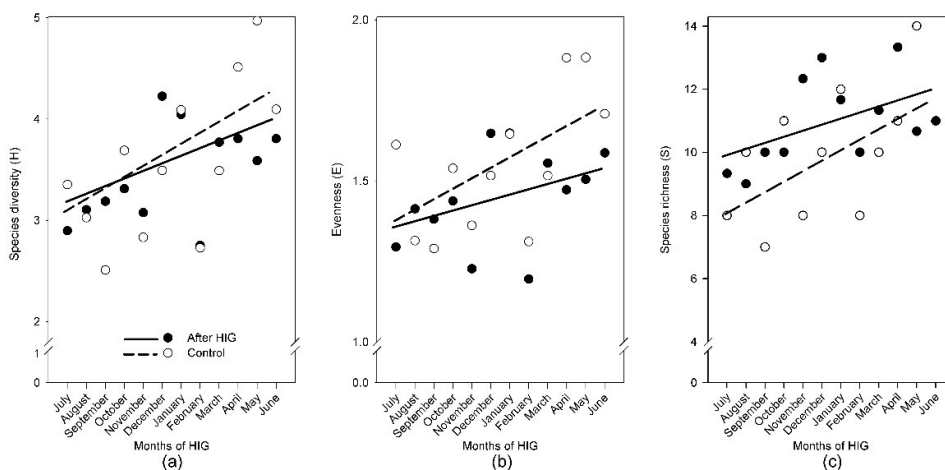


Figure 3. (a) Species diversity (H), (b) Evenness (E) and (c) Richness (S) monitored for the period of one year comparing HIG and control plots. At each sampling time, HIG was applied exactly one year ago. The regression analysis indicated that the slopes are not different ($p < 0.05$) between HIG and the control sites for the three diversity parameters.

4. Discussion

Previous results showed that HIG as a management practice in this sub-tropical grassland was able to reduce standing dead biomass, thereby increasing the green biomass share and promoting grass growth [26]. However, these positive effects for livestock farming need to be considered with the respective interests of sustainable land use practices and diversity conservation. In general, the idea of

using pulse grazing as a management tool generated a strong controversial debate about benefits and risks [41–43]. One major concern with regard to the Northern Argentinean natural grasslands is the possible effect on plant species composition resulting from such a strong impact.

However, our study showed that the diversity parameters of species richness, H, E and S did not differ between grasslands subjected to HIG compared to the control. Also, the seasonal impact timing, which might be of importance due to the different phenological growth phases and seasonal climate conditions, did not affect the diversity parameters. The same results with no difference between HIG and control sites were derived from a monthly analysis over the course of one year (Figure 3A–C). The observed trend of simultaneously increasing diversity parameters for the HIG and control sites during the analyzed time period must thus be addressed to a superordinated process triggering this (natural) vegetation dynamic.

In ecological theory, an HIG grazing event can be considered a strong ecosystem disturbance that should promote plant species following a ruderal strategy (R-strategy) (sensu [44], C-S-R theory). Therefore, possible shifts in species composition in response to HIG might counteract the positive effects of SDB reduction. The higher quality fodder species found in the Chaco grasslands mainly belong to the functional group following a more competitive (C) or stress tolerant (S) strategy; both are considered to be relatively sensitive to a strong disturbance. HIG could therefore negatively affect plant species diversity, grassland productivity and quality by triggering shifts in plant species composition [44]. According to our results, concerns about a shift to more plant species following a ruderal strategy in response to HIG disturbance were not confirmed. HIG did not alter the green cover dominance of the rather competitive *Poaceae* species and did not reduce the *Fabaceae* species cover. However, we found a clear increase of *Cyperaceae*, while the cover of *Asteraceae* species decreased; the latter are in general considered of poor palatability or even toxic for cattle, as *Senecio grisebachii* and *Baccharis coridifolia* toxic for ruminants [7,45,46]. Encroachment by small trees, forbs or shrubs is a major threat to both grassland productivity and diversity in the Chaco region [5,6]. Examples of invasive species are *Prosopis* sp. [5], or most frequently other species belonging to the *Asteraceae* family. Therefore, HIG could have the potential to contribute to a progressive de-encroachment of natural grasslands.

HIG favored an increase of C₄ plant cover, this functional group likely profits most from more transmission of light due to less SDB [47,48]. However, regarding fodder quality, the C₄ grasses have lower digestibility than C₃ species [49]. Complementarily, C₃ species represent only 5–8% of the cover in sub-tropical Argentina [50]. After HIG we recorded a strong increase of C₃ species green biomass cover. To date, there was no previous report of such a management-induced increase of grassland C₃ species [50], which will likely translate into increased fodder quality. Further analyses of HIG effects on fodder quality might therefore be highly interesting for the livestock production. Nevertheless, not all C₃ species are palatable; particularly non-desirable is the perennial C₃ species *Eringium horridum* Malme, which possesses trampling resistant rosettes, which grow in summer. The *E. horridum* individuals show a great capacity to resprout after plant damage [51]. The PCA reveals the magnitude of the effects of HIG on grassland vegetation. Control is ascribed to C₃ and SDB. HIG timed in spring is likely to produce a grassland with higher diversity and a higher proportion of annual species. Conversely, HIG timed in autumn, winter or summer is associated with an increase in perennials and monocots, which means foraging species for cows (Figure 2). Therefore, timed HIG used as pulse disturbance could improve diversity or increase grass foraging. However, we are aware that HIG could produce delayed responses affecting diversity that were not captured during our two years of observation. Therefore, our results should be carefully considered in the context of long-term effects. HIG as a management option is developing as a serious alternative in the context of sustainable land use intensification. Although HIG management may increase the herding efforts, the benefits that are generated exceed the additional effort required.

5. Conclusions

High impact grazing showed no effect on the diversity parameters species richness, Shannon–Wiener diversity index (H) and the Shannon’s equitability index (E). The plant functional groups C₃ and C₄ monocotyledonous and perennials increased their green biomass cover after HIG. The biomass cover of species belonging to the *Asteracea* family was reduced after HIG. Used as pulse disturbance, HIG has the potential to function as an alternative management practice towards sustainable land use intensification and the reduction of “low value” species encroachment in the Chaco grassland ecosystem. Our results suggest that HIG is not shifting plant species composition to a more ruderal plant community, but instead promotes previously established rather competitive and higher value fodder species. Nevertheless, we are aware that there is a need to explore the effects of HIG on fodder quality.

The newly generated information helps to provide a better understanding of the effects of pulse disturbances on the diversity of the rangeland system in Northern Argentina. The biggest barrier to its adoption, as an alternative—on farm—management option, maybe culture and tradition. In this area, farmers may consider that HIG management might require an additional planning and herding effort. Nevertheless, when applied in a timely manner, it contributes to the sustainable use of grassland resources. The present study offers the opportunity, not only for farmers, but also for the extension services to better support farm-scale decisions that maintain the naturally rich and diverse grasslands.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Table A1. Detailed list of all grassland plant species sampled in the experimental area at the Corrientes National Institute of Agriculture Research Station, at peak biomass time during February 2014.

Species	Family	¹ Number of Cotyledons	² Life Cycle	³ Photo-Synthesis Pathway	⁴ Response to HIG
<i>Justicia laevilinguis</i> (Nees) Lindau	Acanthaceae	2	Pe	C ₄	i
<i>Ruellia</i> sp. L.	Acanthaceae	2	An	C ₄	i
<i>Alternanthera philoxeroides</i> (Mart.) Griseb.	Amaranthaceae	2	Pe	C ₄	in
<i>Amaranthus</i> sp. L.	Amaranthaceae	2	An	C ₄	i
<i>Gomphrena celocoides</i> Mart.	Amaranthaceae	2	An	C ₄	d
<i>Zephyranthes</i> sp. Herb.	Amaryllidaceae	1	Pe	C ₃	i
<i>Eryngium coronatum</i> Hook. & Arn.	Apiaceae	2	Pe	C ₃	i
<i>Eryngium ebracteatum</i> Lam.	Apiaceae	2	Pe	C ₃	i
<i>Eryngium horridum</i> Urb. ex H. Wolff, Malme	Apiaceae	2	Pe	C ₃	i
<i>Ammis majus</i> L.	Apiaceae	2	An	CAM	i
<i>Apium leptophyllum</i> (Pers) F. Muell. Ex Benth	Apiaceae	2	Pe	CAM	i
<i>Aristolochia fimbriata</i> Cham.	Aristolochiaceae	2	Pe	C ₃	i
<i>Asclepias curassavica</i> L.	Asclepiadaceae	2	Pe	C ₃	i
<i>Acmella</i> sp. R.K. Jansen	Asteraceae	2	An	C ₄	i
<i>Baccharis coridifolia</i> DC.	Asteraceae	2	Pe	C ₄	i
<i>Baccharis notorsegila</i> Griseb.	Asteraceae	2	Pe	C ₄	d
<i>Baccharis punctulata</i> DC.	Asteraceae	2	Pe	C ₄	in
<i>Baccharis</i> sp. DC.	Asteraceae	2	Pe	C ₄	i
<i>Bidens pilosa</i> L.	Asteraceae	2	An	C ₄	in
<i>Chaptalia nutans</i> (L.) Polák	Asteraceae	2	Pe	C ₄	i
<i>Conyza bonariensis</i> (L.) Cronquist	Asteraceae	2	An	C ₄	i
<i>Eupatorium candolleum</i> Hook. & Arn.	Asteraceae	2	Pe	C ₄	i
<i>Eupatorium macrocephalum</i> Less.	Asteraceae	2	Pe	C ₄	d
<i>Eupatorium clematideum</i> Griseb.	Asteraceae	2	Pe	C ₄	in
<i>Eupatorium subhastatum</i> Hock. & Arn.	Asteraceae	2	Pe	C ₄	i
<i>Eupatorium</i> sp. Hock. & Arn.	Asteraceae	2	Pe	C ₄	in
<i>Mikania coridifolia</i> (L.f.) Willd.	Asteraceae	2	Pe	C ₄	in
<i>Orthopappus angustifolius</i> (Sw.)	Asteraceae	2	Pe	C ₄	d
<i>Pterocaulon lorentzii</i> Malme	Asteraceae	2	An	C ₄	in
<i>Pterocaulon pycnostachyum</i> (Michx.) Elliott	Asteraceae	2	Pe	C ₄	in
<i>Pterocaulon</i> sp. DC.	Asteraceae	2	Pe	C ₄	d
<i>Senecio grisebachii</i> Baker	Asteraceae	2	Pe	C ₄	i

Table A1. Cont.

Species	Family	¹ Number of Cotyledons	² Life Cycle	³ Photo-Synthesis Pathway	⁴ Response to HIG
<i>Solidago chilensis</i> Meyen	Asteraceae	2	Pe	C ₄	i
<i>Vernonia incana</i> (Less.) DC.	Asteraceae	2	Pe	C ₄	i
<i>Vernonia chamaedryes</i> Lees.	Asteraceae	2	Pe	C ₄	i
<i>Vernonia rubricaulis</i> Hum. & Bonpl.	Asteraceae	2	Pe	C ₄	d
<i>Chenopodium nigra</i> L.	Chenopodiaceae	2	An	C ₄	i
<i>Commelina erecta</i> L.	Commelinaceae	1	Pe	CAM	i
<i>Tripogandra radiata</i> (C.B. Clarke) Bacigalupo	Commelinaceae	1	Pe	CAM	i
<i>Dichondra repens</i> J.R. Forst. & G. Forst.	Convolvulaceae	2	Pe	C ₄	i
<i>Evolvulus sericeus</i> Sw.	Convolvulaceae	2	Pe	C ₄	d
<i>Ascolepis brasiliensis</i> (Kunth) Benth. Ex Clarke.	Cyperaceae	1	Pe	C ₄	i
<i>Carex sororia</i> Kunth	Cyperaceae	1	Pe	C ₄	i
<i>Cyperus aggregatus</i> (Willd.) Endl.	Cyperaceae	1	Pe	C ₃	in
<i>Cyperus entrerrianus</i> Boeckeler	Cyperaceae	1	Pe	C ₄	i
<i>Cyperus haspan</i> ssp. <i>Juncooides</i>	Cyperaceae	1	Pe	C ₃	i
<i>Cyperus iria</i> L.	Cyperaceae	1	Pe	C ₄	in
<i>Cyperus obtusatus</i> (J. Presl & C. Presl) Mattf. & Kük.	Cyperaceae	1	Pe	C ₄	i
<i>Cyperus rigens</i> C. Presl	Cyperaceae	1	Pe	C ₄	i
<i>Cyperus virens</i> Michx.	Cyperaceae	1	Pe	C ₄	i
<i>Eleocharis nodulosa</i> (Roth) Schult.	Cyperaceae	1	Pe	C ₄	i
<i>Eleocharis viridans</i> Kük ex. Osten	Cyperaceae	1	Pe	C ₄	i
<i>Fimbristylis dichotoma</i> (L.) Vahl	Cyperaceae	1	Pe	C ₄	i
<i>Rhynchospora corymbosa</i> (L.) Britton	Cyperaceae	1	Pe	C ₄	in
<i>Rhynchospora scutellata</i> Griseb.	Cyperaceae	1	Pe	C ₄	i
<i>Rhynchospora tenuis</i> Link	Cyperaceae	1	Pe	C ₄	i
<i>Scleria sellowiana</i> Kunth	Cyperaceae	1	Pe	C ₄	d
<i>Pteridium aquilinum</i> (L.) Kuhn	Dennstaedtiaceae	-	Pe	C ₃	d
<i>Scoparia dulcis</i> L.	Escrofulariaceae	2	An	C ₃	i
<i>Scoparia muricata</i> L.	Escrofulariaceae	2	An	C ₃	in
<i>Phyllanthus stipulatus</i> (Raf.) G.L. Webster	Euphorbiaceae	2	An	C ₄	i
<i>Euphorbia prostrata</i> Aiton	Euphorbiaceae	2	An	C ₄	d
<i>Tragia geraniifolia</i> Klotzsch ex Müll.Arg.	Euphorbiaceae	2	Pe	C ₄	i
<i>Aeschynomene americana</i> L.	Fabaceae	2	Pe	C ₃	i
<i>Chamaecrista rotundifolia</i> (Pers.) Greene	Fabaceae	2	Pe	C ₃	d
<i>Desmanthus virgatus</i> (L.) Wild.	Fabaceae	2	Pe	C ₃	i
<i>Desmodium pachyrizum</i> Vogel	Fabaceae	2	Pe	C ₃	i
<i>Rhynchosia laterita</i> Burkart	Fabaceae	2	Pe	C ₃	in

Table A1. Cont.

Species	Family	¹ Number of Cotyledons	² Life Cycle	³ Photo-Synthesis Pathway	⁴ Response to HIG
<i>Desmanthus depressus</i> Willd.	Fabaceae	2	Pe	C ₃	in
<i>Desmodium incanum</i> DC.	Fabaceae	2	Pe	C ₃	i
<i>Discolobium</i> sp. Benth.	Fabaceae	2	Pe	C ₃	in
<i>Galactia marginalis</i> Benth.	Fabaceae	2	An	C ₃	i
<i>Indigofera asperifolia</i> Benth.	Fabaceae	2	Pe	C ₃	i
<i>Leucaena leucocephala</i> (Lam.) de Wit	Fabaceae	2	Pe	C ₃	i
<i>Macroptilium lathyroides</i> (L.) Urb.	Fabaceae	2	Pe	C ₃	d
<i>Macroptilium postratum</i> Benth. (Urb.)	Fabaceae	2	An	C ₃	d
<i>Phaseolus</i> sp. Benth.	Fabaceae	2	Pe	C ₃	in
<i>Rhynchosia edulis</i> Griseb.	Fabaceae	2	Pe	C ₃	i
<i>Rhynchosia</i> sp. Lour.	Fabaceae	2	Pe	C ₃	in
<i>Stylosanthes hippocampoides</i> Mohlenbr.	Fabaceae	2	Pe	C ₃	i
<i>Stylosanthes montevidensis</i> Vogel	Fabaceae	2	Pe	C ₃	in
<i>Hydrolea spinosa</i> L.	Hydrophyllaceae	2	An	C ₃	i
<i>Sisyrinchium</i> sp. Baker	Iridaceae	1	Pe	C ₃	d
<i>Juncus microcephalus</i> Kunth	Juncaceae	1	Pe	C ₃	i
<i>Hyptis lappacea</i> Benth.	Labiadae	2	An	C ₃	i
<i>Nothoscordum inodorum</i> (Aiton) G. Nicholson	Liliaceae	1	Pe	C ₃	in
<i>Selaginella</i> sp. Spring	Lycopodiopsida	-	Pe	C ₃	i
<i>Cuphea carthagenensis</i> (Jacq.) J. F. Macbr.	Lythraceae	2	Pe	C ₃	i
<i>Cuphea lysimachioides</i> Cham. & Schltldl.	Lythraceae	2	Pe	C ₃	i
<i>Cuphea</i> sp. Koehne	Lythraceae	2	Pe	C ₃	i
<i>Heymia salicifolia</i> (Kunth) Link & Otto	Lythraceae	2	Pe	C ₃	i
<i>Krapovickasia</i> sp. Fryxell	Malvaceae	2	An	C ₃	i
<i>Malvastrum coromandelianum</i> (L.) Garcke	Malvaceae	2	An	C ₃	i
<i>Melochia hernanioides</i> A. St. Hil.	Malvaceae	2	Pe	C ₃	i
<i>Sida rhombifolia</i> L.	Malvaceae	2	Pe	C ₃	i
<i>Sida tuberculata</i> R.E.Fr.	Malvaceae	2	Pe	C ₃	i
<i>Marsilea consinea</i> Mirb.	Marsiliaceae	-	Pe	C ₃	i
<i>Cissampelos</i> sp. Kunth	Menispermaceae	2	Pe	C ₃	i
<i>Ludwigia major</i> (Micheli) Ramamoorthy	Onagraceae	2	Pe	C ₃	i
<i>Orchidia</i> sp. Juss.	Orchidaceae	1	Pe	CAM	in
<i>Oxalis</i> sp. L.	Oxalidaceae	2	Pe	CAM	i
<i>Passiflora coerulea</i> L.	Passifloraceae	2	Pe	CAM	i
<i>Plantago officinalis</i> Crantz	Plantaginaceae	2	Pe	CAM	i
<i>Andropogon lateralis</i> Ness	Poaceae	1	Pe	C ₄	i

Table A1. Cont.

Species	Family	¹ Number of Cotyledons	² Life Cycle	³ Photo-Synthesis Pathway	⁴ Response to HIG
<i>Axonopus affinis</i> Chase	Poaceae	1	Pe	C ₄	in
<i>Axonopus compressus</i> (Sw.) P. Beauv.	Poaceae	1	Pe	C ₄	i
<i>Axonopus fissifolius</i> (Raddi) Kuhlman	Poaceae	1	Pe	C ₄	i
<i>Bothriochloa laguroides</i> DC.	Poaceae	1	Pe	C ₄	i
<i>Bothriochloa saccharoides</i> Sw.	Poaceae	1	Pe	C ₄	i
<i>Briza uniolae</i> (Nees) Steud.	Poaceae	1	An	C ₄	i
<i>Chloris distichophylla</i> Lag.	Poaceae	1	Pe	C ₄	i
<i>Cynodon dactylon</i> (L.) Pers.	Poaceae	1	Pe	C ₄	i
<i>Digitaria insularis</i> (L.) Mez ex Ekman	Poaceae	1	Pe	C ₄	in
<i>Digitaria phaeotrix</i> (Trin.) Parodi	Poaceae	1	Pe	C ₄	i
<i>Digitaria</i> sp. Haller	Poaceae	1	An	C ₄	i
<i>Eleusine indica</i> (L.) Gaertn.	Poaceae	1	Pe	C ₄	i
<i>Eleusine tristachya</i> Lam	Poaceae	1	Pe	C ₄	i
<i>Elyonurus muticus</i> (Spreng.) Kuntze	Poaceae	1	An	C ₄	i
<i>Eragrostis airoides</i> Nees	Poaceae	1	Pe	C ₄	i
<i>Eragrostis bahiensis</i> Roem. & Schult.	Poaceae	1	Pe	C ₄	i
<i>Hemarthria altissima</i> (Poir) Stapf & C.E. Hubb.	Poaceae	1	Pe	C ₄	i
<i>Leersia hexandra</i> Sw.	Poaceae	1	Pe	C ₃	i
<i>Panicum miliaceum</i> L.	Poaceae	1	Pe	C ₄	i
<i>Panicum milioides</i> Ness. Ex Trin.	Poaceae	1	Pe	C ₄	i
<i>Panicum prionitis</i> Nees	Poaceae	1	Pe	C ₄	-
<i>Panicum</i> sp. L.	Poaceae	1	Pe	C ₄	in
<i>Paspalum acuminatum</i> Raddi	Poaceae	1	Pe	C ₄	i
<i>Paspalum almun</i> Chase	Poaceae	1	Pe	C ₄	i
<i>Paspalum notatum</i> Flügé	Poaceae	1	Pe	C ₄	i
<i>Paspalum plicatulum</i> Michx.	Poaceae	1	Pe	C ₄	i
<i>Paspalum simplex</i> Morong	Poaceae	1	Pe	C ₄	i
<i>Paspalum urvillei</i> Steud.	Poaceae	1	Pe	C ₄	i
<i>Phalaris</i> sp. Jansen & Wacht.	Poaceae	1	An	C ₄	i
<i>Piptochaetium montevidense</i> (Spreng.) Parodi	Poaceae	1	Pe	C ₄	in
<i>Rotboellia selloana</i> L.F.	Poaceae	1	Pe	C ₄	i
<i>Schizachyrium microstachyum</i> (Desv.) Roseng.	Poaceae	1	Pe	C ₄	i
<i>Setaria geniculata</i> P.Beauv.	Poaceae	1	Pe	C ₄	i
<i>Sorghastrum pellitum</i> (Hack.) Parodi	Poaceae	1	Pe	C ₄	in
<i>Sorghastrum setosum</i> (Griseb.) Hitchc.	Poaceae	1	Pe	C ₄	i
<i>Sporobolus indicus</i> (L.) R. Br.	Poaceae	1	Pe	C ₄	i

Table A1. Cont.

Species	Family	¹ Number of Cotyledons	² Life Cycle	³ Photo-Synthesis Pathway	⁴ Response to HIG
<i>Sporobolus monandrus</i> Roseng., B.R. Arrill. & Izag.	Poaceae	1	Pe	C ₄	in
<i>Sporobolus poiretii</i> (Roem. & Schult.) Hitchc.	Poaceae	1	Pe	C ₄	i
<i>Sporobolus</i> sp. R. Br.	Poaceae	1	Pe	C ₄	i
<i>Steinchisma hians</i> (Elliott) Nash	Poaceae	1	Pe	C ₄	i
<i>Steinchisma laxa</i> Sw.	Poaceae	1	Pe	C ₄	i
<i>Tridens brasiliensis</i> (Steud.) Parodi	Poaceae	1	Pe	C ₃	i
<i>Polygala molluginifolia</i> A. St. Hil.	Polygalaceae	2	An	C ₃	i
<i>Polygala obovata</i> A. St. Hil. & Moq.	Polygalaceae	2	An	C ₃	i
<i>Polygala</i> sp. DC.	Polygalaceae	2	An	C ₃	i
<i>Muehlenbeckia sagittifolia</i> (Ortega) Meisn.	Polygonaceae	2	Pe	C ₄	i
<i>Poligonum punctatum</i> Elliot	Polygonaceae	2	Pe	C ₃	i
<i>Clematis bonariensis</i> Juss. Ex DC.	Ranunculaceae	2	Pe	C ₃	i
<i>Relbunium richardianum</i> (Gillies ex Hook. & Arn.) Hicken	Rubiaceae	2	An	C ₃	d
<i>Rubiaceae</i> Juss.	Rubiaceae	2	An	C ₃	d
<i>Bouchetia anomale</i> (Miers) Britton & Ruby	Solanaceae	2	Pe	C ₃	i
<i>Petunia</i> sp. Juss.	Solanaceae	2	An	C ₃	i
<i>Phisalis viscosa</i> L.	Solanaceae	2	Pe	C ₃	i
<i>Solanum granulosum-leprosum</i> Dunal	Solanaceae	2	Pe	C ₃	in
<i>Turnera sidoides</i> DC.	Turneraceae	2	An	C ₃	d
<i>Lantana</i> sp. Moldenke	Verbenaceae	2	Pe	C ₃	d
<i>Lippia</i> sp. Moldenke	Verbenaceae	2	Pe	C ₃	d
<i>Glandularia peruviana</i> (L.) Small	Verbenaceae	2	Pe	C ₃	i
<i>Glandularia rigida</i> Sprengel	Verbenaceae	2	Pe	C ₃	i
<i>Verbena litoralis</i> Kunth	Verbenaceae	2	Pe	C ₃	i
<i>Verbena rigida</i> Spreng.	Verbenaceae	2	Pe	C ₃	i

¹ Number of cotyledons: 1 = Monocotyledons, 2 = Dicotyledons; ² Life cycle: An = Annual, Pe = Perennial; ³Photosynthesis pathway: CAM = Crassulacean acid metabolism and C₃ or C₄ species.

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