



The Dilemma of Improving Native Grasslands by Overseeding Legumes: Production Intensification or Diversity Conservation[☆]



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ABSTRACT

In native campos of Uruguay, overseeding legumes coupled with phosphorus (P) fertilization is a technology used to increase animal production. Short-term improvements in both forage productivity and quality are repeatedly reported. However, some evidence suggests that this management may at times lead to the collapse of the native community and invasions by exotic species. Indeed, it is yet unclear to what extent overseeding legumes into native grasslands affects its long-term integrity. This study uses data from a long-term experiment to assess whether increased P fertilizer rates—typically used to encourage legume establishment and growth—are associated with reduced species diversity. In 1996 a grazed native grassland in eastern Uruguay was either left untouched (control) or overseeded with a mix of *Trifolium repens* and *Lotus corniculatus* and then fertilized at either a moderate or high rate of P (197 or 394 kg · ha⁻¹ over 13 years, respectively). The three treatments were arranged in a randomized block design with four replicates of 2 hectares each. In 2005 the experiment was exhaustively sampled: 11 georeferenced sampling points per replicate, each encompassing ~20 m². Extractable P was measured in the 0–5- and 5–15-cm soil layers. In 2009, species presence and cover were measured at the same points. Across treatments, wherever legumes were introduced, extractable soil P was negatively related to species richness and diversity ($P < 0.01$) and native grass cover was reduced. This effect became asymptotic once soil P exceeded 27 and 36 mg · kg⁻¹ of P (0–5 cm), respectively. Therefore the documented reduction in species richness and diversity suggests a trade-off between increased pasture production and decreased vegetation stability may be operating in response to P fertilization of overseeded grasslands. The underlying ecophysiological mechanisms, as well as grazing management options to mitigate species diversity decline, should be further studied.

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Introduction

Overseeding legumes coupled with phosphorus (P) fertilization is an effective procedure to establish high-quality forage species into native grasslands and has been adopted in many agro-ecological regions of the world (Coates et al., 1990; Cook, 1980; Risso and Berretta, 1997; Tiecher et al., 2014). In the short term, this technology improves the quantity of forage, prevents protein deficiency in the diet of animals, and thus increases livestock productivity (Ferreira et al., 2011; Risso and Berretta, 1997; Soca et al., 2002). This practice also replaces the

need for nitrogen (N) fertilization (Bryan, 1985). However, the limited persistence of legumes (Muir et al., 2011) and frequent invasions of exotic species (Tognetti and Chaneton, 2012) are a relevant long-term concern, from both agronomic management and ecological conservation points of view. Low-legume persistence in overseeded grasslands emphasizes the need to identify management thresholds that prevent alternative vegetation states dominated by exotic invaders. At the same time, it is important to know how different species and communities respond to soil nutrient enrichment if we are to conserve grassland biodiversity (Isbell et al., 2013).

There is a growing interest to understand the impact of management practices on ecosystem services of native grasslands (Carvalho and Batello, 2009; Paruelo and Vallejos, 2013). For example, in the campos biome region of South America, native grasslands represent the largest agro-ecosystem and provide valuable economic and ecosystem services. These ecosystems are part of the Río de la Plata grasslands, representing

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a unique biodiversity reserve with more than 3 000 species of temperate and subtropical plants (Bilenca and Miñarro, 2004). However, they are critically threatened by changes in land use (Overbeck et al., 2007). Native grasslands are in general stable and persistent, but livestock production with traditional management is economically limited, driving changes in land use to forestry or crop production. Therefore sustainable management of these grasslands requires reconciling environmental conservation and production priorities (Bullock et al., 2001; Lemaire, 2012).

Variability of climatic and soil conditions in the Río de la Plata grasslands is the main driver that favors the existence of highly diverse plant communities with differing growth strategies. Species diversity can stabilize productivity and prevent exotic species invasions through multiple strategies to acquire resources (Dukes, 2001; Hector et al., 2010; Hooper et al., 2005) and greater use of limiting factors (Hooper and Dukes, 2004). However, it has been found that fertilization weakens the positive effects of diversity on stability (Hautier et al., 2014; Suding et al., 2005), especially at local scales (Di Tommaso and Aarssen, 1989), and increases the probability of system collapse and subsequent evolution to low-diversity states. The fluctuating resources theory of invasibility postulates that rapid increases in resources supplies makes a plant community more susceptible to exotic species invasion (Davis et al., 2000). In this case dominant invasive species may act as transformer species, modifying both diversity and function (Richardson et al., 2000) and changing the state of the grassland community.

Production limitations in the campos biome ecosystems are mainly related to climatic variability (Bettolli et al., 2010) and deficiencies of N and P content in soils (Pallares et al., 2005). It is recognized that soil P controls the growth and persistence of forage legumes in native grasslands (Coates et al., 1990; Pallares et al., 2005). Nevertheless, there are concerns about adverse effects of P enrichment on the diversity of species adapted to N and P limited soils (Ceulemans et al., 2011). The aim of this study was to determine whether increased P fertilizer rates are associated with reduced species richness and diversity in native grasslands in which legumes were overseeded. We hypothesize that increased levels of soil P concentrations from P additions aimed at encouraging legume establishment and growth led to 1) reductions in species richness and diversity and 2) increases in the abundance of exotic invasive species.

Methods

Study Area

The study was carried out in the “Palo a Pique” experimental farm, located in Eastern Uruguay (33°15'44"S, 54°28'59"W; 50 m above sea level). This region is part of the “Río de la Plata grasslands,” a biome of temperate and subtropical grasslands dominated by C4 and C3 perennial grasses. It extends from East Central Argentina to Southern Brazil and includes most of Uruguay (Soriano, 1991). At the experimental site, mean annual rainfall varies between 1000 and 1600 mm, evenly distributed throughout the year, and mean monthly temperature varies from 11°C in winter to 23°C in summer. The topography is softly hilly (slope 2–3%), and soils are a mixture of Abruptic Argiaquolls and Oxyaquic Vertic Arguidolls (fine, smectitic, and thermic) (Durán et al., 2005). The most abundant species are C4 grasses, such as *Axonopus fissifolius* (Raddi) Kuhl, *Paspalum notatum* Flügge, *Aristida murina* Cav., *Andropogon ternatus* (Spreng.) Nees, and *Paspalum plicatulum* Michx., which averaged a dry matter (DM) production of 3400 kg · ha⁻¹ · yr⁻¹ over a 12-yr period (Bermúdez and Ayala, 2005). The main soil characteristics of the study site are presented in Table 1.

Experimental Design and Treatments

The experiment was established in 1996 in a completely randomized block design with four replications. The three treatments consisted

Table 1

Major soil characteristics of native and overseeded grasslands in the year 2005 (0–15 cm)

	Native Grasslands	Legume-Overseeded Grasslands
pH (1:1 soil:water)	5.5	5.5
Organic C (%)	2.1	2.2
Extractable P citric acid (mg · Kg ⁻¹)	3.3	22.6
Extractable K (meq · 100 g ⁻¹)	0.27	0.27
Clay (g · Kg ⁻¹)	180	160
Sand (g · Kg ⁻¹)	510	560

of native grasslands (NG) and native grassland broadcasted with a mixture of 4 kg · ha⁻¹ of *Trifolium repens* cv. Zapican and 8 kg · ha⁻¹ of *Lotus corniculatus* cv. San Gabriel (each one inoculated with their specific *Rhizobium*) and then fertilized with either a moderate (MP) or high rate of P (HP). The MP received 19.6 kg P · ha⁻¹ · yr⁻¹ at sowing and then 13.1 kg P · ha⁻¹ · yr⁻¹, while the HP received 39.3 kg P · ha⁻¹ · yr⁻¹ and then 26.2 kg P · ha⁻¹ · yr⁻¹. During the first 3 years, superphosphate was used (21% P₂O₅ soluble—23% P₂O₅ total). Later, rock phosphate (0% P₂O₅ soluble—29% P₂O₅ total) was used. All experimental units (12 paddocks in total, of 2 hectares each) were rotationally grazed by yearling steers and heifers. Paddocks were grazed with similar moderate grazing intensities among treatments. In a previous study, Palacio (1999) reported forage allowances of 8.3 and 13.0 (kg of DM · 100 kg of animal live weight) for legume-overseeded treatments and NG, respectively. Average above-ground standing biomass ± standard errors from March 2008 to March 2010 were 2 802 ± 194 kg DM · ha⁻¹, 3412 ± 193 kg DM · ha⁻¹ and 3578 ± 183 kg DM · ha⁻¹, for NG, MP, and HP, respectively.

Soil Sampling and Analysis

Soils were sampled in the spring of 2005 in 11 georeferenced points regularly distributed within each paddock (132 sampling points in total). At each sampling point, within a 2.5-m radius, eight 2.5-cm diameter sampling cores were taken from the 0- to 5-cm and 5- to 15-cm soil depths. Soil samples were then dried at 45°C for 72 h, ground, and sieved to pass through a 2-mm sieve. Extractable phosphorus content was measured adapting the citric acid method proposed by Thompson (1995). Fifty milliliters of citric acid (0.5%) were added to 5 g of soil and stirred for 30 minutes. The extract was filtered through a Whatman No. 2 filter paper. A 5-mL aliquot of filtered extract was removed and mixed with 8 mL of colorimetric reagent (Murphy and Riley, 1962) and then diluted to 50 mL. The blue color development was measured after 45 minutes by absorbance at 882 nm with a UV spectrometer (Thermo Spectronic, Genesys 10S) and compared with known reference concentrations. Soil texture was evaluated by a modification of the Bouyoucos hydrometer method with gravimetric determination of the sand content (Beretta et al., 2014).

Vegetation Sampling

Plant species composition was measured in the late spring of 2009 using a modified Braun-Blanquet abundance scale as proposed by Mueller-Dombois and Ellenberg (1974). In the exact same 11 georeferenced points per replicate where soil had been sampled, the name of all vascular plant species present was recorded and their aerial cover (when > 5%) was visually estimated in 1-m² quadrats. Species-area curves determined previously (not shown here) confirmed that 11 m² of sampling area was adequate to represent the community species richness. Above-ground net primary pasture production was seasonally estimated from March 2008 to March 2010 using two exclusion cages (1 × 0.5 m) per paddock (moved after each cut) according to the method described by Klingman et al. (1943). At the beginning of every season of the year herbage was clipped to ground level. At the

end of each season, all accumulated above-ground standing plant material was clipped again in two 0.1 m² quadrats per cage to estimate the forage production over the growth period. The sampled biomass was dried at 60°C for at least 72 h and weighed to determine the DM content.

Data Analysis

Species cover and concentration of extractable soil P of the 132 sampling points constituted the primary database. Six sampling points were removed from the database because their extractable soil P in the 0–5-cm layer was higher than two standard deviations from the mean of the treatment and thus considered outliers. Species richness, native species richness, native grasses species richness, and the Shannon Index (Shannon and Weaver, 1949) were calculated for every sampling unit. The mean seasonal pasture production—expressed as daily growth rate (kg DM · ha⁻¹ · d⁻¹) to compare different periods—was calculated for every paddock. The Shapiro–Wilks test was used to check the assumption of normally distributed residuals for all response variables, and square root transformation was used when necessary. General linear mixed models were performed for each response variable considering block and sampling units nested within treatments as random factors, while treatments were considered a fixed factor. Several models combining different functions for correlations and variance of errors were tested. Next, the best-fitted models for each response variable were selected on the basis of the Akaike Criterion. Means for the significant fixed effects were compared using Fisher's least significant difference (LSD) test ($P < 0.05$). Orthogonal contrasts (control vs. overseeded grasslands and MP vs. HP) were performed to compare species richness and diversity, as well as cover of the main species and functional groups. To examine changes in species composition, a principal component analysis was used. Extractable soil P at both depths and functional group cover (native warm season perennial grasses, native cool season perennial grasses, native species nongrasses, exotic legumes, exotic winter annual grasses, and exotic warm season perennial grasses) were the studied variables. We analyzed only the first two principal component axes because they explained a large proportion of the variation in species composition. Afterward, to identify the occurrence of changes in vegetation state related to the experimental treatments, a discriminant analysis of the dominant functional groups cover was performed. Segmented regressions (Toms and Lesperance, 2003) were used to describe the relationship between richness and diversity versus extractable soil P concentrations:

$$\text{if } x < R, \quad y_{ij} = L + U(R-x) + e_{ij} \quad (1)$$

and

$$\text{if } x > R, \quad y_{ij} = L + e_{ij} \quad (2)$$

where y_{ij} is the dependent variable, x is the independent variable, e_{ij} represents the experimental error, R is the break point, L is the mean value of the zero slope segment, and U indicates the magnitude of the non-zero slope segment. R values are referred to as the thresholds of extractable soil P concentration beyond which losses of richness and diversity become negligible. Statistical analyses were performed using Infostat (Di Rienzo et al., 2015) and JMP (SAS Institute, Cary, North Carolina, USA) software packages.

Results

Continued applications of P fertilizers from 1996 to 2005 increased extractable soil P by 25 and 50 mg · kg⁻¹ in the 0–5 cm layer, and by 0.7 and 3 mg · kg⁻¹ in the 5–15 cm layer, in the MP and HP treatments, respectively (Fig. 1A). These values reflect both a substantial increase and stratification of the distribution of P availability for plants: clearly, resource supply increased faster than the capacity of the vegetation to

use it, and the system was becoming progressively enriched in P. Forage production of overseeded and P-fertilized grasslands was higher than native grasslands across all growing seasons except in autumn 2009. The average forage production of overseeded and P-fertilized grasslands was 60% higher than native grasslands, while the P fertilization level had no significant effect. The maximum differences between overseeded and P-fertilized grasslands with native grasslands were found in the periods of elevated growth (spring 2009, summers 2008–2009 and 2009–2010), with increments in forage production between 69% and 84% (Fig. 2).

A total of 156 different species were recorded in the experimental area. There were significant differences when comparing the native grasslands versus the overseeded and P-fertilized grasslands and between fertilization treatments. In the overseeded grasslands, exotic grasses displaced native species and overseeded legumes. Both cool and warm season native perennial grass cover, as well as native nongrass cover, decreased in overseeded grasslands (Fig. 1B–1D). The legume abundance of the grasslands was generally low, although legume cover was higher in overseeded grasslands than native grasslands (2.5% vs. 0.25%). Overseeded grasslands were dominated by two exotic grasses, the C4 perennial *Cynodon dactylon* and the C3 annual *Lolium multiflorum*, which accounted for an average 68% of cover in overseeded grasslands but only 2.5% in native grasslands. Higher P fertilization resulted in higher cover of exotic species (61% in MP vs. 75% in HP). Overseeded grasslands had lower diversity, species richness, native species richness, and native grass species richness than natural grasslands (Fig. 3). All of these variables were further reduced at the higher P fertilization rate. The most negative effects of the high P fertilization treatment were recorded in native species richness and native grasses richness, which had less than one-third and one-fifth of the native grassland species, respectively. The overseeded grasslands had higher exotic species richness that partially compensated the reduction in native species.

Species diversity and richness had negative relationships with extractable soil P concentration in the 0–5-cm layer (Fig. 4A and B). Segmented regression analysis indicated P thresholds of 36 and 27 mg · kg⁻¹ beyond which the minimum values of 1.1 of the Shannon–Weaver diversity index and 13.9 species per m⁻² were reached, respectively. Species diversity and richness followed the same trend in the 5–15-cm layer, but this relationship was weaker than in the 0–5-cm depth.

Principal component analysis ordination based on the cover of dominant species, functional groups, and extractable soil P concentration also indicates close relationships of extractable soil P with species composition (Fig. 5). The first principal component explained 72% of variation and neatly separated native from overseeded grasslands. The second component explained 11% of the variation and separated warm season from cool season species (i.e., *Cynodon dactylon* from *Lolium multiflorum* and the oversown legumes in overseeded grasslands) and warm season from cool season native perennial grasses in native grasslands.

The biplot of communities' ordination by discriminant analysis summarizes in a simple manner the patterns of variation in species composition. This analysis confirms that native differ from overseeded grasslands, however the fertilization level did not significantly influence the state of overseeded communities (Fig. 6).

Discussion

This study revealed that higher extractable soil P concentrations in the surface soil of native grasslands overseeded with legumes were associated with lower species richness and diversity. Native perennial grasses, were particularly sensitive: after 13 years, their cover decreased by two-thirds. After a decade, this space was not occupied by the overseeded legumes, as legumes were almost absent in all treatments. Instead, two exotic species increased in relative cover: a C3 annual

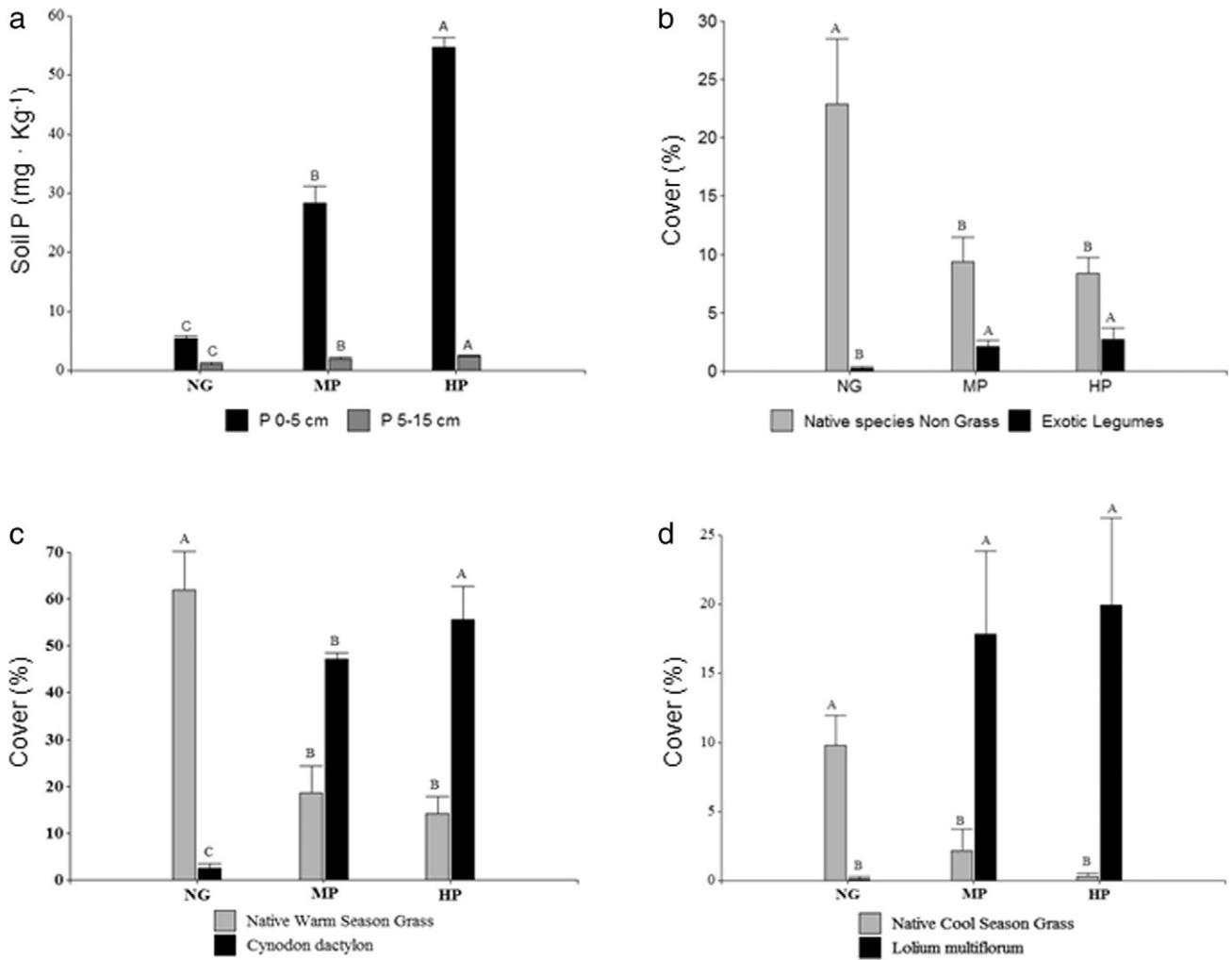


Fig. 1. Treatment effects on A) extractable soil P in the 0–5 cm and 5–15 cm layer; B) native species nongrass and exotic legumes; C) native warm season grass and *Cynodon dactylon*; D) native cool season grass and *Lolium multiflorum*. HP, high-phosphate, legume-overseeded grassland; MP, moderate-phosphate, legume-overseeded grassland; NG, natural grassland. Different letters within each parameter (columns within the same color) indicate significant differences ($P < 0.05$), and bars represent the standard error.

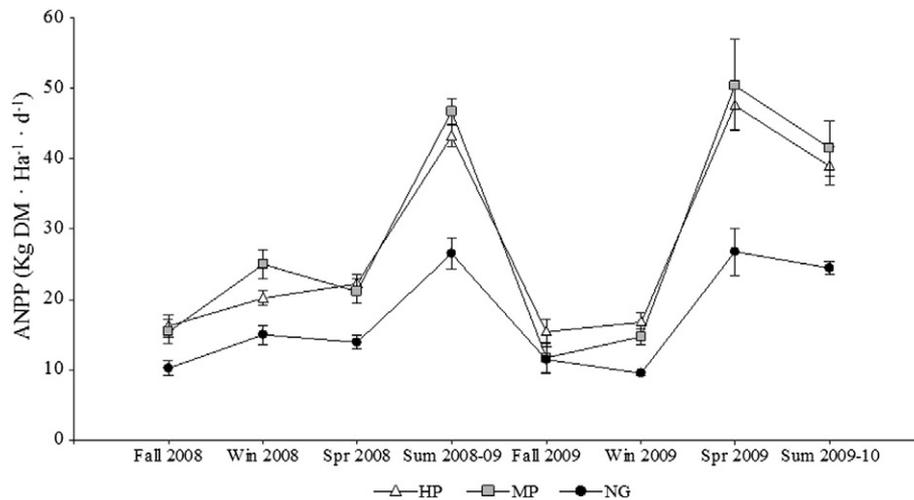


Fig. 2. Seasonal variation in aboveground net primary production (ANPP) grassland dry matter (DM) production from March 2008 to March 2010. Each point represents the daily mean growth ($\text{kg DM} \cdot \text{ha}^{-1}$) of the treatment in each season. HP, high-phosphate, legume-overseeded grassland (triangles); MP, moderate-phosphate, legume-overseeded grassland (squares); NG, native grassland (circles).

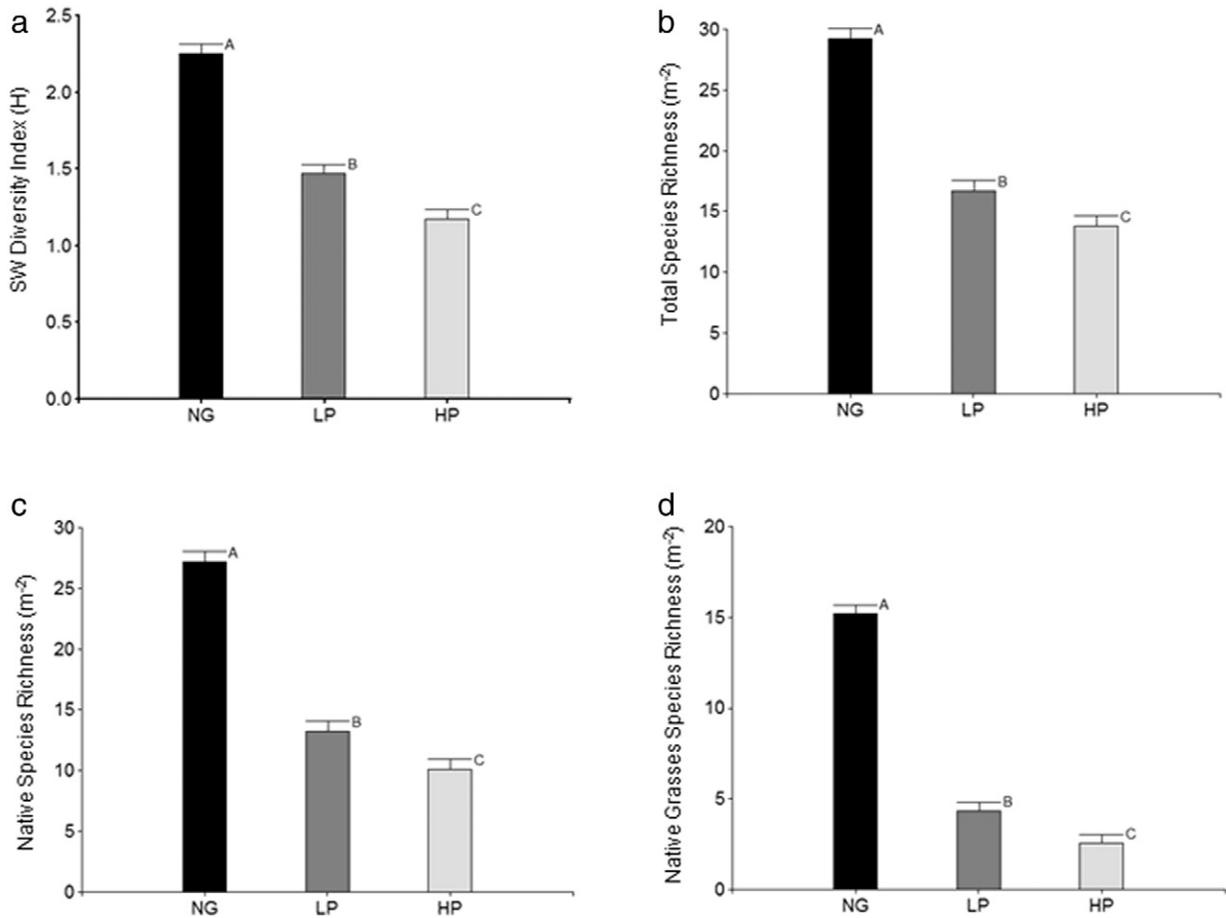


Fig. 3. Treatment effects on **A)** Shannon–Weaver diversity index; **B)** total species richness; **C)** native species richness; and **D)** native grasses species richness. HP, high-phosphate legume overseeded grassland; MP, moderate-phosphate, legume-overseeded grassland; NG, natural grassland. Different letters between treatments correspond to significant differences ($P < 0.05$), and bars represent the standard error.

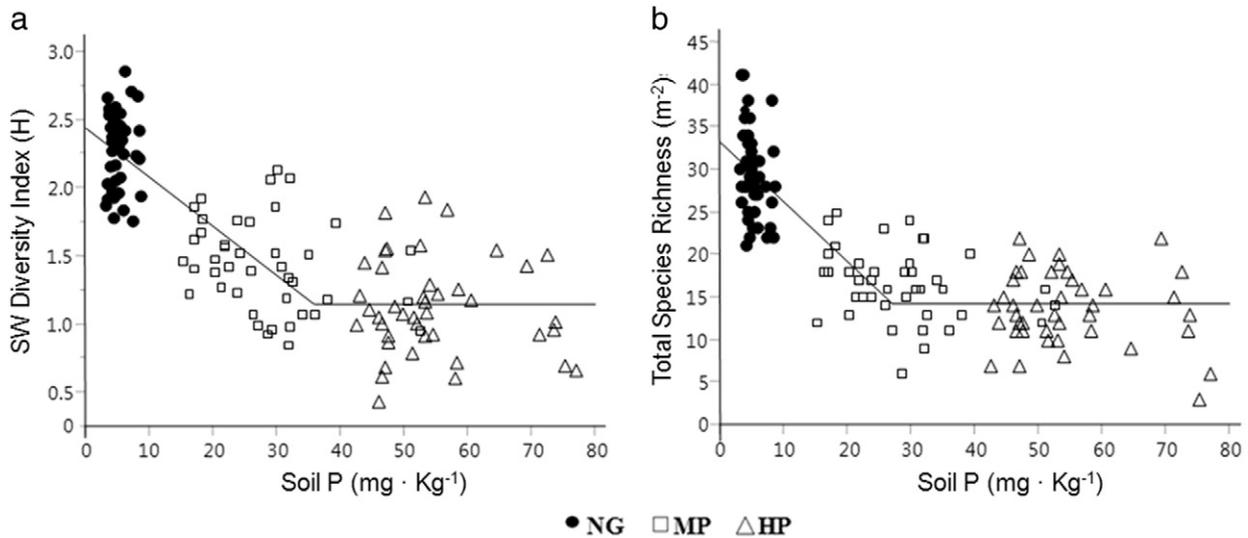


Fig. 4. Relationships between extractable soil P concentrations ($\text{mg} \cdot \text{kg}^{-1}$) with **A)** Shannon Weaver Index ($H = 1.11 + 0.037 \cdot (35.9 - P)$ if $P < 35.9$ and $H = 1.11$ if $P > 35.9$, $R^2 = 0.67$, $P < 0.0001$, root mean square error (RMSE) = 0.34; **B)** total species richness ($R = 13.6 + 0.73 \cdot (27.0 - P)$ if $P < 27.0$ and $R = 13.6$ if $P > 27.0$, $R^2 = 0.69$, $P < 0.0001$, RMSE = 4.60). The 126 sampling units surveyed consist of native grasslands (circles); moderate P-fertilized, legume-overseeded grassland (squares); and high P-fertilized, legume-overseeded grassland (triangles).

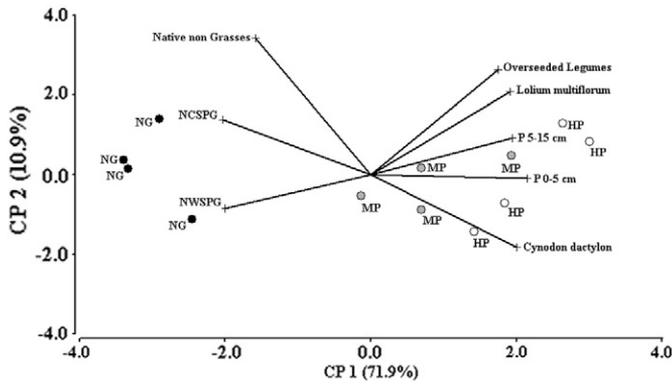


Fig. 5. Biplot of principal components analysis considering the principal species or groups of species cover and soil characteristics. The arrows represent the direction of the main effects, and the circles summarize each paddock position in the multivariate space. Treatment: HP, high-phosphate, legume-overseeded grassland; MP, moderate-phosphate, legume-overseeded grassland; NG, native grassland. Cover of: NWSPPG, native warm season perennial grasses; NCSPPG, native cool season perennial grasses; nongrasses, native species nongrasses; legumes, overseeded legumes; *Lolium*, *Lolium multiflorum*; *Cynodon*, *Cynodon dactylon*. Soil characteristics: P = mg · kg⁻¹ in the depths of: 0–5 and 5–15 cm.

ryegrass (*Lolium multiflorum*) and a C4 stoloniferous perennial grass (*Cynodon dactylon*).

Potential negative effects of P availability on native communities have been described repeatedly, for instance, in seminatural grasslands of Europe (Gilbert et al., 2009; Janssens et al., 1998) and by Lambers et al. (2010) in ancient landscapes of western Australia. The latter authors reported that species-rich communities are absent in soils with high P concentrations. Likewise, Ceulemans et al. (2013) identified that soil P is a better predictor of plant species richness than soil N and concluded that P enrichment is a threat to plant diversity. However, the actual ecophysiological mechanism(s) by which P enrichment affected native species in the overseeded grasslands of the present study are not known. Multiple, nonmutually exclusive alternatives are possible.

It is well established that legume establishment and growth are encouraged by P fertilization both in native overseeded grasslands (Risso and Berretta, 1997) and sown grasslands (Bermúdez et al. 2008). The cause, it is believed, is related to the limiting levels of plant-available P and nitrogen that these soils have (Pallares et al., 2005). Legumes, having the P restriction eased by fertilization, become highly competitive due to their ability to fix atmospheric nitrogen. Previous work in our

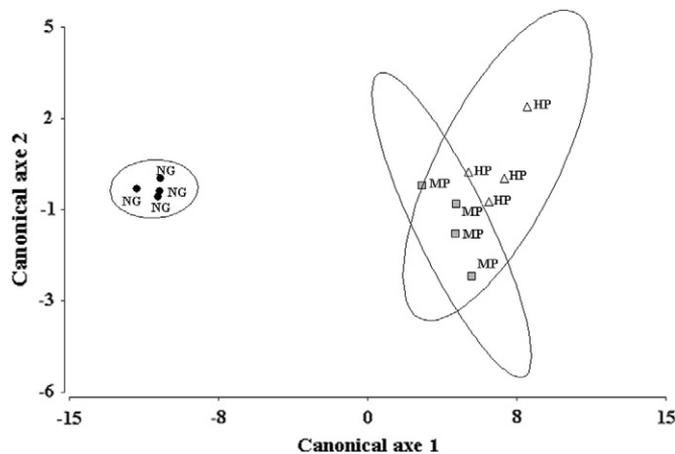


Fig. 6. Graphic representation of discriminant analysis considering the principal species or groups of species cover. The symbols summarize each paddock position in the multivariate space. Treatments: HP, high-phosphate, legume-overseeded grassland (triangles); MP, moderate-phosphate, legume-overseeded grassland (squares); NG, native grassland (circles). Ellipses show the prediction interval with a probability of 95%.

study site reported 31% and 69% of legume cover in MP and HP, respectively, in the third year of the experiment (Palacio, 1999), revealing the dominance of overseeded species at this stage. Some evidence indeed indicates that these legumes were actively fixing N in these grasslands (Mallarino and Wedin, 1990).

The reasons for the limited persistence of the legumes are even less certain. It may be that sustained progressive enrichment of soil N due to biological N fixation makes legumes less competitive and thus grasses dominate. A model of self-regulation was proposed by Chapman et al. (1996) to explain the temporal/spatial variations in legume content of ryegrass/white clover swards based on the amount of mineral N in the soil. Nevertheless, this dynamic response of grasses and legumes to N availability also indicates that it is unlikely to maintain high legume contents in native grasslands if soils are simultaneously N and P enriched. Another possibility is that drought events drastically reduced legume persistence, leaving empty gaps in the sward (Acuña et al., 2012). In any case, the grasses that took advantage of the reduced legume cover were not the original native species. However, two exotics, the C3 winter-growing *L. multiflorum*, which has a high response to N (Vallano et al., 2012), and the C4 summer-growing *C. dactylon*, which has large ability to explore the soil (Lobet et al., 2012), became dominant.

Several functional mechanisms have been proposed to explain fertilization effects on species richness and diversity. One regularly hypothesized mechanism is a shift from below-ground competition for nutrients to above-ground competition for light as the environment becomes richer in any limiting resource (Goldberg and Miller, 1990; Hautier et al., 2009). In the third year of the experiment, the forage production was on average 101% higher in overseeded and P-fertilized grasslands than native grasslands (Palacio, 1999). In addition, after a decade, the forage production was on average 60% higher in overseeded and P-fertilized grasslands than native grasslands. The increased production would be one of the main causes of species losses in overseeded and P-fertilized grasslands, but it is unknown if native species richness resulted from the additive or interactive effects of root and shoot competition. Mycorrhizal–plant interactions also influence how P availability affects native species (Van der Heijden et al., 1998). Pezzani et al. (2012) found lower root mycorrhizal colonization in the natives grasses *Steinchisma hians* and *Mnesithea selleana* when present in legume-overseeded and P-fertilized grasslands than in native grassland. Interestingly, *Cynodon dactylon* showed high levels of mycorrhizal colonization in both situations.

The observed relationship between P availability and species richness and identity in overseeded grasslands led us to propose a graphical state and transition model depicted in Fig. 7. State and transition models are useful tools for grasslands management (Knapp et al., 2011; Laycock, 1991; Westoby et al., 1989). The proposed model consists of two alternative steady states (native grassland and exotic grass–invaded grassland) and an intermediate unsteady state characterized by (native

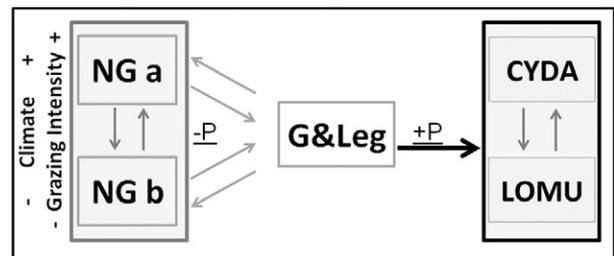


Fig. 7. A conceptual state and transition model proposed for long-term evolution of overseeded and P-fertilized grasslands. Gray arrows are community pathways, the black arrow is the transition, and gray boxes are the steady states. In the cases of native grasslands, the community phases a and b are related to grazing or climate events. In the exotic-invaded grasslands, the *Cynodon dactylon* (CYDA) or *Lolium multiflorum* (LOMU) dominance is related to the season of the year. The at-risk grassland community is the legume-overseeded grassland (G & Leg), and the transition is controlled mainly by soil P concentration.

grasses plus overseeded legumes) “the at-risk community” whose duration would be related to legume persistence. Then, the return to a “native grassland” state or the transition to a state “dominated by exotic grasses” would depend on soil P concentration and native species abundance. This first approach needs further research to establish state and transition framework recommendations (Briske et al., 2008). Despite the lower diversity, the grasslands invaded by exotics grasses were more productive than native grasslands, particularly in springs and summers with good growing conditions, but it is not known what the response might be in periods of drought.

Willems and van Nieuwstadt (1996) and Isbell et al. (2013) have already shown that a low-diversity, exotic-dominated state may persist decades after N or P enrichment ceases, and Tognetti and Chaneton (2012) showed that reestablishment of native perennial grasses was constrained by species like *Cynodon dactylon* and *Lolium multiflorum*, which prevent recovery from the soil seed bank.

It is noteworthy that all of HP and half of MP sampling units exceed the P threshold beyond which diversity and richness appear to remain at consistent lower levels. Although we do not know whether the condition observed at every sampling point was in a steady state or evolving toward another condition, the asymptotic shape of the relationship—virtually no change in species composition above extractable soil P of $\sim 30 \text{ mg} \cdot \text{kg}^{-1}$ —suggests a new vegetation state may have been reached after the 13 years of the experiment. The validity of this hypothesis should be the aim of subsequent studies. Specifically, because this study did not include soils between 6 and $18 \text{ mg} \cdot \text{kg}^{-1}$ of extractable P, the possibility of long-term coexistence of a diverse array of native species with overseeded legumes cannot be ruled out, especially, when the likelihood of strong interactions between P availability and grazing management is high (Schwinning and Parsons, 1996).

Implications

The negative relationship documented by the present study between species diversity and the concentration of extractable soil P in native grasslands overseeded with legumes suggests that a trade-off between increased forage production and quality versus reduced integrity of the plant community may be operating. Neither the underlying ecophysiological mechanisms nor whether this response is modified by grazing management is known and should therefore be the subject of further investigation. Our findings do indicate that, first, a precautionary approach is warranted when considering the level of P fertilization, and second, soil testing to quantify extractable soil P must be a standard part of the agronomic management to avoid unnecessary inputs and to prevent diversity losses. Therefore the trade-off between short-term economic response and long-term sustainability should be taken into account considering the ecological limits of the intensification.

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References

Acuña, H., Inostroza, L., Tapia, G., 2012. Strategies for selecting drought tolerant germplasm in forage legume species. In: Rahman, I., Hasegawa, H. (Eds.), *Water stress*. InTech, Rijeka, Croatia, pp. 277–300.

Beretta, A.N., Silbermann, A.V., Paladino, L., Torres, D., Bassahun, D., Musselli, R., García-Lamohte, A., 2014. Soil texture analyses using a hydrometer: modification of the Bouyoucos method. *Ciencia Invest. Agrar.* 41, 263–271.

Bermúdez, R., Ayala, W., 2005. Producción de forraje de un campo natural de la zona de lomadas del este. In: Seminario de actualización técnica en manejo de campo natural. INIA, Montevideo, Uruguay, pp. 33–40.

Bermúdez, R., Ayala, W., Carámbula, M., Morón, A., 2008. Fuentes fosfatadas en la rotación soja-pasturas en lomadas del este. Seminario de actualización técnica en fertilización fosfatada de pasturas en la región este. INIA, pp. 111–118.

Bettolli, M.L., Altamirano, M.A., Cruz, G., Rudorff, F., Martínez, A., Arroyo, J., Armoa, J., 2010. Pastura natural de Salto (Uruguay): relación con la variabilidad climática y análisis de contextos futuros de cambio climático. *Rev. Bras. Meteorol.* 25, 248–259.

Bilenca, D., Miñarro, F., 2004. Identificación de Áreas Valiosas de Pastizal (AVPs) en las Pampas y Campos de Argentina, Uruguay y sur de Brasil. Fundación Vida Silvestre, Buenos Aires (353 pp.).

Briske, D.D., Bestelmeyer, B.T., Stringham, T.K., Shaver, P.L., 2008. Recommendations for development of resilience-based state-and-transition models. *Rangel. Ecol. Manag.* 61, 359–367.

Bryan, W.B., 1985. Effects of sod-seeding legumes on hill land pasture productivity and composition. *Agron. J.* 77, 901–905.

Bullock, J.M., Franklin, J., Stevenson, M.J., Silvertown, J., Coulson, S.J., Gregory, S.J., Tofts, R., 2001. A 12-year grazing experiment on species-poor grassland: vegetation responses and correlation with plant traits. *J. Appl. Ecol.* 38, 253–267.

Carvalho, P.C., Batello, C., 2009. Access to land, livestock production and ecosystem conservation in the Brazilian Campos biome: the natural grasslands dilemma. *Livest. Sci.* 120, 158–162.

Ceulemans, T., Merckx, R., Hens, M., Honnay, O., 2011. A trait based analysis of the role of phosphorus vs nitrogen enrichment in plant species loss across Northwest European grasslands. *J. Appl. Ecol.* 48, 1145–1163.

Ceulemans, T., Merckx, R., Hens, M., Honnay, O., 2013. Plant species loss from European semi natural grasslands following nutrient enrichment—is it nitrogen or is it phosphorus? *Glob. Ecol. Biogeogr.* 22, 73–82.

Chapman, D.F., Parsons, A.J., Schwinning, S., 1996. Management of clover in grazed pastures: expectations, limitations and opportunities. *Spec. Publ. Agron. Soc. N. Z.* 11, 55–64.

Coates, D.B., Kerridge, P.C., Miller, C.P., Winter, W.H., 1990. Phosphorus and beef production in northern Australia. 7. The effect of phosphorus on the composition, yield and quality of legume-based pasture and their relation to animal production. *Trop. Grasslands* 24, 209–220.

Cook, S.J., 1980. Establishing pasture species in existing swards: a review. *Trop. Grasslands* 14, 181–187.

Davis, M.A., Grime, J.P., Thompson, K., 2000. Fluctuating resources in plant communities: a general theory of invasibility. *J. Ecol.* 88, 528–534.

Di Rienzo, J.A., Casanoves, F., Balzarini, M.G., Gonzalez, L., Tablada, M., Robledo, C.W., 2015. InfoStat versión 2015 [computer program] Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Córdoba, Argentina.

Di Tommaso, A., Aarssen, L.W., 1989. Resource manipulation in natural vegetation: a review. *Vegetatio* 84, 9–29.

Dukes, J.S., 2001. Biodiversity and invasibility in grassland microcosms. *Oecologia* 126, 563–568.

Durán, A., Califra, A., Molfino, J.H., Lynn, W., 2005. Keys to soil taxonomy for Uruguay. U.S. Department of Agriculture, Natural Resources Conservation Service (77 pp.).

Ferreira, E.T., Nabinger, C., Elejalde, D.A.G., Freitas, A.K.D., Carassai, I.J., Schmitt, F., 2011. Fertilization and oversowing on natural grassland: effects on pasture characteristics and yearling steers performance. *Rev. Bras. Zootec.* 40, 2039–2047.

Gilbert, J., Gowing, D., Wallace, H., 2009. Available soil phosphorus in semi-natural grassland: assessment methods and community tolerances. *Biol. Conserv.* 142, 1074–1083.

Goldberg, D.E., Miller, T.E., 1990. Effects of different resource additions of species diversity in an annual plant community. *Ecology* 71, 213–225.

Hautier, Y., Niklaus, P.A., Hector, A., 2009. Competition for light causes plant biodiversity loss after eutrophication. *Science* 324 (5927), 636–638.

Hautier, Y., Seabloom, E.W., Borer, E.T., Adler, P.B., Harpole, W.S., Hector, A., 2014. Eutrophication weakens stabilizing effects of diversity in natural grasslands. *Nature* 508, 521–525.

Hector, A., Hautier, Y., Saner, P., Wacker, L., Bagchi, R., Joshi, J., Loreau, M., 2010. General stabilizing effects of plant diversity on grassland productivity through population asynchrony and overyielding. *Ecology* 91, 2213–2220.

Hooper, D.U., Dukes, J.S., 2004. Overyielding among plant functional groups in a long-term experiment. *Ecol. Lett.* 7, 95–105.

Hooper, D.U., Chapin, F.S., Ewell, J.J., Hector, A., Inchausti, P., Wardle, D.A., 2005. Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecol. Monogr.* 75, 3–35.

Isbell, F., Tilman, D., Polasky, S., Binder, S., Hawthorne, P., 2013. Low biodiversity state persists two decades after cessation of nutrient enrichment. *Ecol. Lett.* 16, 454–460.

Janssens, F., Peeters, A., Tallowin, J.R.B., Bakker, J.P., Bekker, R.M., Fillat, F., Oomes, M.J.M., 1998. Relationship between soil chemical factors and grassland diversity. *Plant Soil* 202, 69–78.

Klingman, D.L., Miles, S.R., Mott, G.O., 1943. The cage method for determining consumption and yield of pasture herbage. *J. Am. Soc. Agron.* 35, 739–746.

Knapp, C.N., Fernandez-Gimenez, M., Kachergis, E., Rudeen, A., 2011. Using participatory workshops to integrate state-and-transition models created with local knowledge and ecological data. *Rangel. Ecol. Manag.* 64, 158–170.

Lambers, H., Brundrett, M.C., Raven, J.A., Hopper, S.D., 2010. Plant mineral nutrition in ancient landscapes: high plant species diversity on infertile soils is linked to functional diversity for nutritional strategies. *Plant Soil* 334, 11–31.

Laycock, W.A., 1991. Stable states and thresholds of range condition on North American rangelands: a viewpoint. *J. Range Manag.* 44, 427–433.

Lemaire, G., 2012. Intensification of animal production from grassland and ecosystem. In: Hemming, D. (Ed.), *Animal science reviews 2012*. CABI, Oxfordshire, UK, pp. 45–52.

- Llobet, M., Vignolio, O.R., Save, R., Biel, C., 2012. Above- and below-ground interactions between *Lotus tenuis* and *Cynodon dactylon* under different fertilization levels. *Can. J. Plant Sci.* 92, 45–53.
- Mallarino, A.P., Wedin, W.F., 1990. Nitrogen fertilization effects on dinitrogen fixation as influenced by legume species and proportion in legume-grass mixtures in Uruguay. *Plant Soil* 124, 127–135.
- Mueller-Dombois, D., Ellenberg, H., 1974. Aims and methods of vegetation ecology. Wiley, New York, NY [547 pp.].
- Muir, J.P., Pitman, W.D., Foster, J.L., 2011. Sustainable, low input, warm season, grass–legume grassland mixtures: mission (nearly) impossible? *Grass Forage Sci.* 66, 301–315.
- Murphy, J., Riley, J.P., 1962. A modified single solution method for the determination of phosphate in natural waters. *Anal. Chim. Acta* 27, 31–36.
- Overbeck, G.E., Muller, S.C., Fidelis, A., Pfadenhauer, J., Pillar, V.D., Blanco, C.C., Boldrini, I.I., Both, R., Forneck, E.D., 2007. Brazil's neglected biome: the South Brazilian Campos. *Perspect. Plant Ecol. Evol. Syst.* 9, 101–116.
- Palacio, R., 1999. Respuesta del campo natural a la siembra de leguminosas y fertilización fosfatada en términos de forraje y producto animal. Trabajo de pasantía, Escuela Agraria "Piraraja.", Lavalleja, Uruguay (18 pp.).
- Pallares, O.R., Berretta, E.J., Maraschin, G.E., 2005. The South American campos ecosystem. In: Suttie, J., Reynolds, S.G., Batello, C. (Eds.), *Grasslands of the world. Food and Agriculture Organization, Rome, Italy*, pp. 171–219.
- Paruelo, J.M., Vallejos, M., 2013. Ecosystem services related to carbon dynamics: its evaluation using remote sensing techniques. In: Alcaraz-Segura, D., Di Bella, C., Straschnoy, J. (Eds.), *Earth observation of ecosystem services. CRC Press Group, Boca Raton, FL, USA*, pp. 17–32.
- Pezzani, F., Del Pino, A., Lezama, F., Rodriguez, A., Parodi, G., García, S., Alchurrut, M., Jaurena, M., 2012. Long-term impacts of phosphorous fertilization on natural grasslands in Uruguay: nutrient contents and arbuscular mycorrhizal status of dominant species. *Proceedings 19th ISTRO Conference, Montevideo, Uruguay*, p. 227.
- Richardson, D.M., Pyšek, P., Rejmánek, M., Barbour, M.G., Panetta, F.D., West, C.J., 2000. Naturalization and invasion of alien plants: concepts and definitions. *Divers. Distrib.* 6, 93–107.
- Risso, D., Berretta, E.J., 1997. Animal productivity and dynamics of native pastures improved with oversown legumes in Uruguay. *Proceedings 18th International Grassland Congress, Winnipeg and Saskatoon, Canada*, pp. 22–30.
- SAS Institute Inc. [JMP software], 2010. SAS Institute Inc., Cary, NC, USA [145 pp.].
- Schwinning, S., Parsons, A.J., 1996. Analysis of the coexistence mechanisms for grasses and legumes in grazing systems. *J. Ecol.* 799–813.
- Shannon, C., Weaver, W., 1949. *The mathematical theory of communication*. University of Illinois Press, Urbana, Urbana, IL, USA [177 pp.].
- Soca, P., Ayala, W., Bermúdez, R., 2002. The effect of herbage allowance of *Lotus pedunculatus* cv. Grasslands Maku on winter and spring beef heifer performance. *Proceedings of a Conference—New Zealand Grassland Association*, pp. 81–84.
- Soriano, A., 1991. Río de la Plata grasslands. In: Coupland, R.T. (Ed.), *Natural grasslands: introduction and Western Hemisphere*. Elsevier, Amsterdam, The Netherlands, pp. 367–407.
- Suding, K.N., Collins, S.L., Gough, L., Clark, C., Cleland, E.E., 2005. Functional- and abundance-based mechanisms explain diversity loss due to N fertilization. *Proc. Natl. Acad. Sci. U. S. A.* 102, 4387–4392.
- Thompson, G.R., 1995. The direct determination of phosphorus in citric acid soil extracts by colorimetry and direct-current plasma emission spectroscopy. *S. Afr. J. Plant Soil* 12, 152–157.
- Tiecher, T., Oliveira, L.B., Rheinheimer, D.S., Quadros, F.L.F., Gatiboni, L.C., Brunetto, G., Kaminski, J., 2014. Phosphorus application and liming effects on forage production, floristic composition and soil chemical properties in the Campos biome, southern Brazil. *Grass Forage Sci.* 69, 567–579.
- Tognetti, P.M., Chaneton, E.J., 2012. Invasive exotic grasses and seed arrival limit native species establishment in an old-field grassland succession. *Biol. Invasions* 14, 2531–2544.
- Toms, J.D., Lesperance, M.L., 2003. Piecewise regression: a tool for identifying ecological thresholds. *Ecology* 84 (8), 2034–2041.
- Vallano, D.M., Selmants, P.C., Zavaleta, E.S., 2012. Simulated nitrogen deposition enhances the performance of an exotic grass relative to native serpentine grassland competitors. *Plant Ecol.* 213, 1015–1026.
- Van der Heijden, M.G., Klironomos, J.N., Ursic, M., Moutoglis, P., Streitwolf-Engel, R., Boller, T., Wiemken, A., Sanders, I.R., 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature* 396, 69–72.
- Westoby, M., Walker, B.H., Noy-Meir, I., 1989. Opportunistic management for rangelands not at equilibrium. *J. Range Manag.* 42, 266–274.
- Willems, J.H., Van Nieuwstadt, M.G.L., 1996. Long-term after effects of fertilization on above-ground phytomass and species diversity in calcareous grassland. *J. Veg. Sci.* 7, 177–184.