The adaptive value of grass traits in response to grazing

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Abstract

Aims

Grazing is associated with several plant traits that may confer resistance to herbivores. However, cross-species analyses do not allow for the differentiation between adaptive evolution and common ancestry. In this study, we evaluated the effect of grazing on 5 morphological traits in 41 native grasses growing in natural grasslands of Uruguay and investigated whether such effects are independent of phylogeny.

Methods

We used data of grass species from 17 paired, grazed and ungrazed plots located in different regions of natural grasslands of Uruguay. For each species, we calculated the Grazing Response Index (GRI) and estimated the culm length, blade length, blade width, blade length/width ratio and caryopsis length. Trait values were calculated as the mean of the maximum and minimum values reported in a public database. We assessed the relationship between the GRI and the morphological traits using cross-species correlations, and we re-examined the correlations using phylogenetically controlled comparative analysis.

Important Findings

Culm length and blade length were significantly correlated with the GRI. Species with higher culms and longer blades diminished their cover under grazing. This association remained significant after statistical control of phylogenetic relatedness among species. By contrast, blade width, blade length/width ratio and caryopsis length did not show any significant relationship with the GRI. Many studies in temperate grasslands recognized that several plant traits respond to grazing but were rarely evaluated in a phylogenetic context. Our results are consistent with the idea that grazing is a selective force with a clear effect on the evolution of grass stature, selecting smaller plants with shorter blades.

Keywords: grasslands, plant height, leaf length, PICs, Uruguay

INTRODUCTION

Grasslands have provided a major food resource for a diverse assemblage of extinct and extant mammal herbivores. The mammalian grazing guild (i.e. consumers of grasses and low forbs characteristically associated with grassland biomes) originated during the first half of the Cenozoic but did not become widespread until the early Miocene (~20–25 million years ago), or perhaps earlier in South America and Africa, associated with the expansion of grass-dominated ecosystems (Jacobs *et al*. 1999; MacFadden 1997). The long periods of uniform environmental conditions, sporadically interrupted by short but highly disruptive periods of change during the tertiary, contrast with the cyclic pattern of climate change that occurred during the quaternary. During the latter, and as a consequence of glacial cycles, cold and dry climates alternated with warmer and wet periods. Accordingly, expansion and retraction of arid (savannas and steppes) and humid (tropical and subtropical forests) biomes have been recorded (Ortiz Jaureguizar and Cladera 2006).

The Rio de la Plata grasslands is one of the largest areas of natural temperate subhumid grasslands in the world, covering more than 700 000 km^2 distributed across eastern Argentina, Uruguay and southern Brazil (Soriano 1992). In this region, and despite the repeated glacial cycles during the Pleistocene, open habitats were well extended, with trees probably restricted to gallery forests following river courses. Since the Pliocene, land mammal fauna was dominated by grazers, with a mix of Patagonian and Central arid taxa with a few subtropical ones (Ortiz Jaureguizar and Cladera 2006). The co-evolution of grasses with large grazing mammals was interrupted 10 000 years ago, when the Pleistocene–Holocene megafaunal

extinction occurred. Since then, natural grasslands evolved under conditions of unknown grazing intensity by native herbivores, mainly the medium-sized (30–40 kg) cervid *Ozotoceros bezoarticus* ("Pampas deer"). Currently, Pampas deer populations are small and highly fragmented, but molecular data and historical records suggest large population sizes until the 19th century (D'Elía 1999; González *et al.* 1998). Grazing by large herbivores was only re-established after the introduction of livestock by the Europeans settlers, about 400 years ago. In Uruguay, current grazing regimes induce changes on community structure and drastically alter plant species composition, increasing species richness (Altesor *et al.* 2005, 2006; Lezama *et al.* 2014). The communities are dominated by prostrate, warm-season (C_4) grasses that are replaced by erect species in grazing exclosures (Altesor *et al.* 2005; Rodríguez *et al.* 2003).

Grazing-induced changes in species composition are accompanied by shifts in grass traits that may confer resistance to grazers and can be considered as functional traits (i.e. phenotypic traits that influence fitness, Reich *et al.* 2003). Grazing favours short-stature species with small leaves and seeds and higher specific leaf area and tillering rate than their counterparts in grazing exclosures (Cingonali *et al.* 2005b; Díaz *et al.* 1992, 2001, 2007; Leoni *et al.* 2009; McNaughton 1984; Noy-Meir *et al.* 1989; Pucheta *et al.* 1992; Sala *et al.* 1986). However, some of these traits allow grasses to cope with arid conditions (Coughenour 1985; Milchunas *et al.* 1988) associated with the expansion of grasslands (Ortiz Jaureguizar and Cladera 2006), making it difficult to determine their original adaptive value.

In order to differentiate phylogenetic effects (or historical reasons) from adaptive changes to current local conditions, phylogenetically controlled comparative methods have been proposed. Studies that incorporate phylogenetic control in their analysis show that some grass traits are evolutionarily related to particular environmental conditions. For example, Villar *et al.* (1998) found that Aegilops species growing in habitats with higher annual rainfall assigned more biomass to shoots than to roots and had higher relative growth rates than species adapted to low-rainfall habitats. Cayssials and Rodríguez (2013) found that narrow and filiform blades are favored in grass species growing in open habitats, while wider and oval blades are favored in species growing in forests.

In this study, we used a phylogenetic framework to evaluate the effects of grazing on 5 morphological traits (culm length, blade length, blade width, blade length/width ratio and caryopsis length) of 41 native grasses growing in natural grasslands of Uruguay. We addressed the following questions: (i) Is there a relationship among morphological traits and the response to grazing across species? (ii) Are these relationships independent of phylogeny and therefore indicating evolutionary convergence?

MATERIALS AND METHODS

Study area

Natural grasslands occupy 68.5% of the total surface of Uruguay (~112 000 km^2, Ministerio de Ganadería & Agricultura

y Pesca 2011) and account for more than 250 species of native grasses (Cayssials and Rodríguez 2013). The climate is temperate, with an annual precipitation of ~1200 mm and a mean monthly temperature of ~17.5°C. Grasslands have been largely devoted to livestock grazing since the European settlement, but during the last two decades, a large proportion of the grassland (~10%) switched to agriculture (mainly soybean) and afforestation.

Species selection and response to grazing

We used data of plant species cover gathered by the Grupo de Ecología de Pastizales (Universidad de la República, Uruguay) from 17 paired, grazed and ungrazed plots located in different regions of natural grasslands of Uruguay. Paired plots were located within the same soil type, and the grazing exclosures were at least 5 years (for sampling details, see Lezama *et al.* 2014). From the list of species, we selected all native grasses that were present in at least three paired plots in order to discard rare species. For each species, we calculated the Grazing Response Index (GRI) of Cingolani *et al.* (2005b), as follows:

$$\text{GRIs} = \frac{\sum_{i=1}^{n} \frac{CGsi - CUsi}{CGsi + CUsi}}{n}$$

where *CGsi* is the total cover of species *s* in the grazed plot of pair *i*, *CUsi* is the total cover of species *s* in the ungrazed plot of the same pair (*i*), and *n* is the number of pairs where the species is present on at least one plot. The GRI index varies between −1 (species that are present only on the ungrazed plots) and +1 (species that are present only on the grazed plots). Values close to 0 indicate the lack of a consistent response of the species to grazing.

Grass traits

The following five morphological traits were considered in this study: (i) culm length, (ii) blade length, (iii) blade width, (iv) blade shape (estimated as the length/width ratio) and (v) caryopsis length. These traits were selected because of their ecological significance in a plant's strategy to cope with energy capture and grazing pressure and because we were able to gather information for the majority of the species considered. Trait values were obtained from Clayton *et al.* (2002 onwards). When the data were missing in the database, we used the information reported by Rosengurtt *et al.* (1970). Midpoint values of the reported ranges were considered.

Phylogenetic relationships

The phylogenetic relatedness among the grass species was determined using the phylogenetic hypothesis for the subfamilies of grasses proposed by the Grass Phylogeny Working Group II (GPWG II) (2012). This phylogeny combines several sets of molecular and morphological data and constitutes the most comprehensive and robust molecular phylogeny for grasses. Using the GPWG II (2012) combined tree as the backbone, we assembled the lately published relationships within the subfamilies Panicoideae (Morrone *et al.* 2012),

Chloridoideae (Peterson *et al*. 2010) and Pooideae (Cialdella *et al*. 2007; Davis and Soreng 2007; Soreng *et al*. 2007).The construction of phylogenetic trees for ecological purposes carries certain difficulties. The sampled species rarely coincide with those used in phylogenetic analyses, making it necessary to substitute taxa based on taxonomic affiliation at the genus and subfamily level (Ackerly and Reich 1999). Taxonomies are suboptimal compared with phylogenetic information but better than assuming that all taxa in a group are equally related (Silvertown and Dodd 1997). Also, uncertainty in the phylogeny at various levels (partly due to the substitution process just mentioned) may lead to numerous polytomies, which potentially reduce the number of contrasts and the power of statistical analyses (Ackerly and Reich 1999).

Grazing response and grass trait relationships

To assess the relationships between the GRI and the five morphological traits, we performed cross-species Pearson correlations analysis using the species trait values (the tips of the phylogenetic tree, TIPs). Since species traits are not completely independent of phylogeny, we also re-examined the correlations using the method of phylogenetically independent contrasts (PICs) developed by Pagel (1992). Each independent contrast represents the difference in the trait values between two related taxa and reflects an independent evolutionary divergence (Ackerly *et al*. 2000). The contrasts are calculated as the standardized differences in trait values and in the GRI between current and/or ancestral pairs of species along the phylogeny. In a fully resolved phylogenetic tree with N original tip species, the number of possible contrasts is $N - 1$, while a phylogenetic tree that includes polytomies produces fewer contrasts. PICs were calculated using the 'crunch' algorithm, implemented in the R package 'CAIC' (Orme *et al*. 2009). We assumed equal length in all branches of the phylogeny. Before analyzing the PICs, we corroborated that the phylogenetic correlations were completely removed (Garland *et al*. 1992). Otherwise, the contrasts were recalculated using logarithmic or square root transformations on the trait values (Freckleton 2000; Purvis and Rambaut 1995). Analyses of PICs correlations were forced through the origin (Purvis and Rambaut 1995). All statistical analyses were done using R (version 2.13.1; R Development Core Team 2011).

RESULTS

Vegetation samples from the 17 pairs of grazed and ungrazed plots included 82 native grasses (~33% of the native grass flora in Uruguayan grasslands). Of these, 41 were present in at least 3 paired plots and were included in the analyses. Twenty-one species showed GRI values between 0.1 and 1.0 and were considered as increasers under grazing regime. Three species belonging to the subfamily Chloridoideae (*Eragrostis neesii*, *Eustachys bahiensis* and *Microchloa indica*) were only registered in the grazed plots (GRI = 1), although they were pre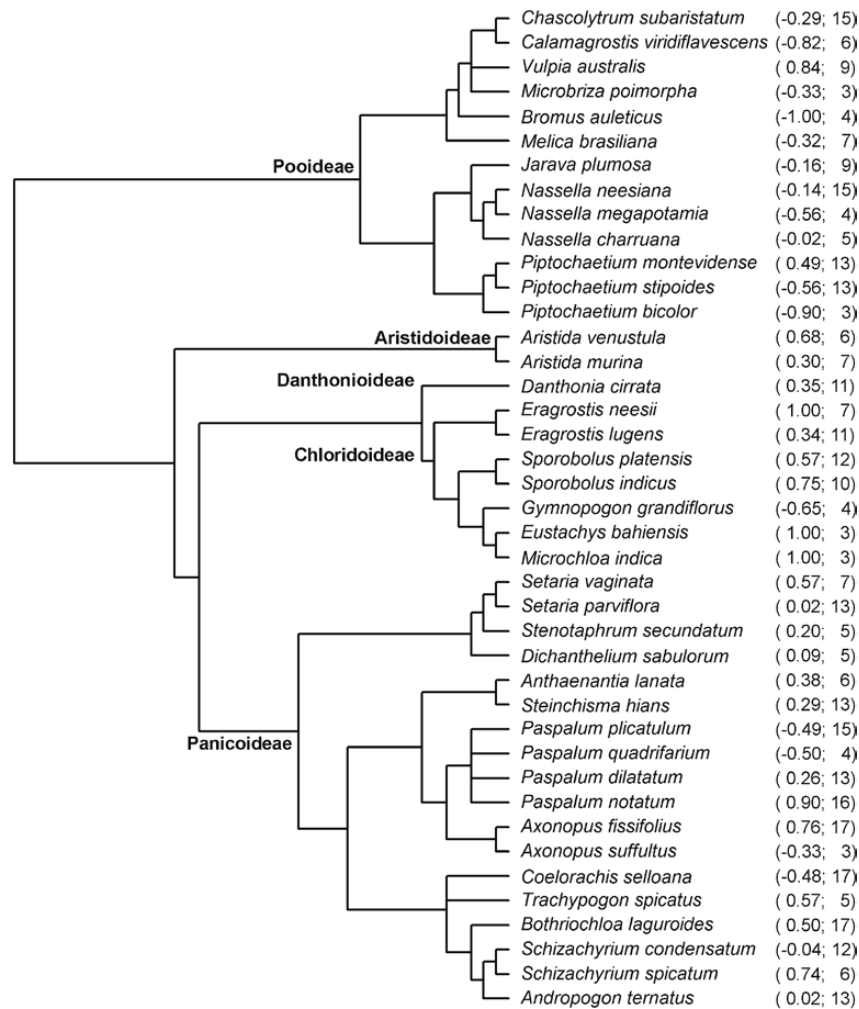sent in few paired plots (3–7). The GRI values of the most frequent and abundant increasers *Axonopus fissifolius* and *Paspalum notatum* (subfamily Panicoideae), ranged between 0.75 and 0.90. On the other hand, 15 species decreased their cover under grazing, with GRI values ranging between −0.1 and −1. *Bromus auleticus* (subfamily Pooideae) was only present in ungrazed plots (GRI = −1). The remaining five species showed GRI values close to 0 and were considered as species inconsistent to grazing (Fig. 1).

The 41 native grasses represented 28 genera and the 5 subfamilies of grasses that compose the Uruguayan grasslands: Pooideae, Aristidoideae, Danthonioideae, Chloridoideae and Panicoideae (Fig. 1). The phylogenetic relatedness among the species was resolved to 90%. The assembled tree had two polytomies for generic relationships and one polytomy at the species level. In consequence, 36 PICs were generated for the vegetative traits from the 40 ($N - 1$) that could be generated in a fully resolved phylogeny. Additionally, due to gaps in information, 31 PICs were generated for the seed length trait (Table 1).

Traits varied by an order of magnitude among species (Table 1). Culm length and blade length were significantly correlated with the GRI. Species with higher culms and longer blades diminish their cover under grazing. This association was not a mere effect of common ancestry; it remained significant after statistical control of phylogenetic relatedness among species. By contrast, blade width, blade length/width ratio and caryopsis length did not show any significant relationship with GRI, neither in the cross-species analyses nor in the phylogenetically controlled analyses (Table 1, Fig. 2).

DISCUSSION

Our results are consistent with the idea that grazing is a major selective force with a clear effect on the evolution of grass stature. The response of the grass species to grazing (estimated through the GRI index) was negatively correlated with the length of the culm and blade. Grass species differ in their resistance to grazing, which is determined by their abilities to regrow after defoliation (tolerance) and to escape grazing (avoidance). Plant height is a morphometric trait related to the ability to avoid grazing (Loreti *et al*. 2001). Under current grazing conditions, Uruguayan grasslands are dominated by prostrate, warm-season (C_4) grasses, and most of the green biomass is concentrated in the 0–10 cm layer (Rodríguez and Cayssials 2011). Indeed, several studies reported reduction in plant height in response to grazing (Diaz *et al*. 2007; McNaughton 1984; Noy-Meir *et al*. 1989; Rodriguez *et al*. 2003). Díaz *et al*. (2001), working with more than a hundred species from temperate grasslands, found that plant height and leaf mass were the traits more clearly associated to grazing. However, these studies did not allow differentiation between adaptive evolution and phylogenetic signal due to the lack of phylogenetic information. In our study, the adaptive value of stature reduction was evidenced in the phylogenetically controlled analysis. McNaughton (1984) noted

	Chascolytrum subaristatum	(−0.29; 15)
	Calamagrostis viridiflavescens	(−0.82; 6)
	Vulpia australis	(0.84; 9)
	Microbriza poimorpha	(−0.33; 3)
	Bromus auleticus	(−1.00; 4)
Pooideae	*Melica brasiliana*	(−0.32; 7)
	Jarava plumosa	(−0.16; 9)
	Nassella neesiana	(−0.14; 15)
	Nassella megapotamia	(−0.56; 4)
	Nassella charruana	(−0.02; 5)
	Piptochaetium montevidense	(0.49; 13)
	Piptochaetium stipoides	(−0.56; 13)
	Piptochaetium bicolor	(−0.90; 3)
Aristidoideae	*Aristida venustula*	(0.68; 6)
	Aristida murina	(0.30; 7)
Danthonioideae	*Danthonia cirrata*	(0.35; 11)
	Eragrostis neesii	(1.00; 7)
	Eragrostis lugens	(0.34; 11)
Chloridoideae	*Sporobolus platensis*	(0.57; 12)
	Sporobolus indicus	(0.75; 10)
	Gymnopogon grandiflorus	(−0.65; 4)
	Eustachys bahiensis	(1.00; 3)
	Microchloa indica	(1.00; 3)
	Setaria vaginata	(0.57; 7)
	Setaria parviflora	(0.02; 13)
	Stenotaphrum secundatum	(0.20; 5)
	Dichanthelium sabulorum	(0.09; 5)
	Anthaenantia lanata	(0.38; 6)
	Steinchisma hians	(0.29; 13)
Panicoideae	*Paspalum plicatulum*	(−0.49; 15)
	Paspalum quadrifarium	(−0.50; 4)
	Paspalum dilatatum	(0.26; 13)
	Paspalum notatum	(0.90; 16)
	Axonopus fissifolius	(0.76; 17)
	Axonopus suffultus	(−0.33; 3)
	Coelorachis selloana	(−0.48; 17)
	Trachypogon spicatus	(0.57; 5)
	Bothriochloa laguroides	(0.50; 17)
	Schizachyrium condensatum	(−0.04; 12)
	Schizachyrium spicatum	(0.74; 6)
	Andropogon ternatus	(0.02; 13)

Figure 1: phylogenetic tree of the 41 native Uruguayan grasses sampled in grazed and ungrazed plots. The tree was assembled following the phylogenetic relationships proposed by GPWG II (2012), Morrone *et al*. (2012), Peterson *et al*. (2010), Davis and Soreng (2007), Soreng *et al*. (2007) and Cialdella *et al*. (2007). Numbers in parentheses indicate the GRIs for each species and the number of paired plots where the species was present (max. = 17).

that grasses can evolve rapidly in response to the prevailing defoliation regime, making grazing an important evolutionary force. The reduction in plant height and leaf length under grazing is an example of the importance of the selective pressures exerted by herbivores in the evolutionary convergence of species attributes.

Blade width and blade width/length ratio were not related to grazing pressure. The width of a leaf is the leaf trait exhibiting the strongest correlation with leaf size (Givnish 1987; Wilson *et al*. 1999) and is regulated independently from the leaf length development (Tsuge *et al*. 1996). Wide-leaved species tend to occupy more shaded environments in order to maximize light interception. High irradiance promotes narrow-leaved forms, associated with drought tolerance (Craine *et al*. 2013). From a set of grasses growing in open (grasslands) and shaded (forest) habitats, Cayssials and Rodríguez (2013) found that blade width and blade width/length ratio were strongly related to environmental conditions. Narrow and filiform blades were favored in species growing in grasslands, and the response was linked to the loss of water and heat. In grasslands, where plants face high radiation loads and high temperature, leaf shapes that minimize their boundary layer (filiform shapes) help to maintain favorable leaf temperatures and higher photosynthetic water use efficiency (Ackerly *et al*. 2002; Givnish and Vermeij 1976; Parkhurst and Loucks 1972). Although grazing affects abiotic conditions (e.g. light availability and temperature, Knapp and Seasted 1986; McNaughton 1979), our results suggest that differences between grazing vs. non-grazing regimes were not enough to create strong divergences in leaf shape.

According to Salisbury (1942), plants in shaded habitats have larger seeds than plants in open habitats. In the absence of grazing, where competition for light becomes significant, larger seeded species would be favored because the seedlings can grow at the expense of reserves, increasing their establishment success and competitive ability. Under

Table 1: morphological traits of native grasses growing in natural grasslands of Uruguay

Trait	Trait values		TIPs				PICs			
	Minimum	Maximum	N	F	df	P	N	F	df	P
Culm length (cm)	12.5	135.0	41	5.266	1, 39	**0.027**	36	7.879	1, 35	**0.008**
Blade length (cm)	3.0	47.5	41	7.683	1, 39	**0.009**	36	5.866	1, 35	**0.021**
Blade width (mm)	0.5	10.0	41	0.541	1, 39	0.466	36	2.715	1, 35	0.108
Blade length/width ratio	6.25	200.0	41	0.289	1, 39	0.594	36	0.112	1, 35	0.739
Caryopsis length (mm)	0.55	8.75	35	0.605	1, 33	0.442	31	0.025	1, 30	0.875

Minimum and maximum values represent the range for all species. Traits were correlated with the species response to grazing (GRI) using the species trait values (TIPs) and PICs. The correlation analyses using independent contrasts were calculated through the origin. Boldface values indicate significance at $P < 0.05$.

grazing conditions, biomass removal and microsite availability increase the colonization opportunities for smaller seeded species (Rees 1996; Rees *et al*. 2001; Turnbull *et al*. 1999; Vesk *et al*. 2004). However, the relationship between grazing and seed size apparently depends upon regional patterns of precipitation (Vesk *et al*. 2004). While the previous hypothesis may be true for subhumid grasslands, where vegetation cover may range between 80% and 100% (Bresciano *et al*. 2014; Díaz *et al*. 1998; Noy-Meir *et al*. 1989), this is not the case for semi-arid or arid environments where the open canopy of these habitats does not site limit recruitment that occurs episodically in response to rainfall (Vesk *et al*. 2004).

In the case of the subhumid Uruguayan grasslands, we expected to find a relationship between the GRI and the caryopsis size. However, this trait was not associated with the grazing regime according to the analyses with and without consideration of phylogeny. This result may be related to the identity of the dominant species in these grasslands. While grazing in subhumid grasslands with short evolutionary history of grazing promotes short annual species (Díaz *et al*. 2007; Milchunas *et al*. 1988), the dominant species under grazing regime in natural grasslands of Uruguay are perennial grasses that allocate little to sexual reproduction, being the vegetative growth through rhizomes and stolons the main mode of propagation (Leoni *et al*. 2009; Rodríguez *et al*. 2003; Rosengurtt 1943). The strategy of horizontal spread would allow the plants to take advantage of the colonization opportunities created by the action of grazers without involving reproductive structures likely to be consumed by herbivores. Moreover, Haretche and Rodríguez (2006) reported a significant shortage of grass seeds in the seed bank of grazed areas of Uruguay. Additionally, using phylogenetic information, Cayssials and Rodríguez (2013) found no habitat effects on the evolution of the caryopsis length from a set of 283 Uruguayan native grasses growing in open (grassland) and shaded (forest) habitats. In this sense, Lord *et al*. (1995) found that a large proportion of the seed size variation in six temperate floras was correlated with the taxonomic membership and therefore the species phylogeny, suggesting phylogenetic conservatism for this trait.

Our results fit the predictions of the generalized model developed by Milchunas *et al*. (1988) for subhumid grasslands with long evolutionary history of grazing. Over evolutionary time, canopy competitiveness and grazing avoidance selected opposite attributes. While the dense canopy of the subhumid grasslands promotes tall species capable of accessing light, grazing is a divergent selective pressure that promotes species of low stature that confers avoidance to herbivores (Milchunas *et al*. 1988). Consequently, subhumid grasslands with a long history of grazing are composed of two sets of species that vary in stature and in their ability to withstand herbivory and are the most responsive communities to grazing. Although the flooding pampa of Argentina (presumably with a similar evolutionary history of grazing than the Uruguayan grasslands) was classified by Milchunas *et al*. (1988) as having short history of grazing, the divergence of adaptations to grazing and canopy competition found in the Uruguayan grasslands suggests that the history of grazing in these grasslands may be longer than assumed by Milchunas *et al*. (1988). Moreover, the low incidence of exotic species in the Uruguayan grasslands (Bresciano *et al*. 2014), the dominance of rhizomatous grasses under grazing and the rapid species turnover after fencing (Rodriguez *et al*. 2003) are other aspects that fit better with a long history of grazing. As Cingolani *et al*. (2005a) pointed out, the evolutionary history of grazing of southern South America is controversial and the estimation of grazing intensity over time should be more precise when it comes to predict responses at a regional or local scale.

In summary, the results of this study are consistent with the idea that herbivores have influenced the evolution of the length of the culm and blade of the Uruguayan native grasses, selecting smaller plants with shorter blades. The coincidence between the analysis of TIPs and PICs indicates that the relationship is independent of phylogeny. Many studies in temperate grasslands recognized several plant trait responses to grazing but were rarely evaluated in a phylogenetic context. In the absence of grazing, a set of tall species replaced prostrate or short-stature grasses widespread under grazing, indicating the presence of divergent selective forces. In contrast with the flooding pampa of Argentina, our results better match the
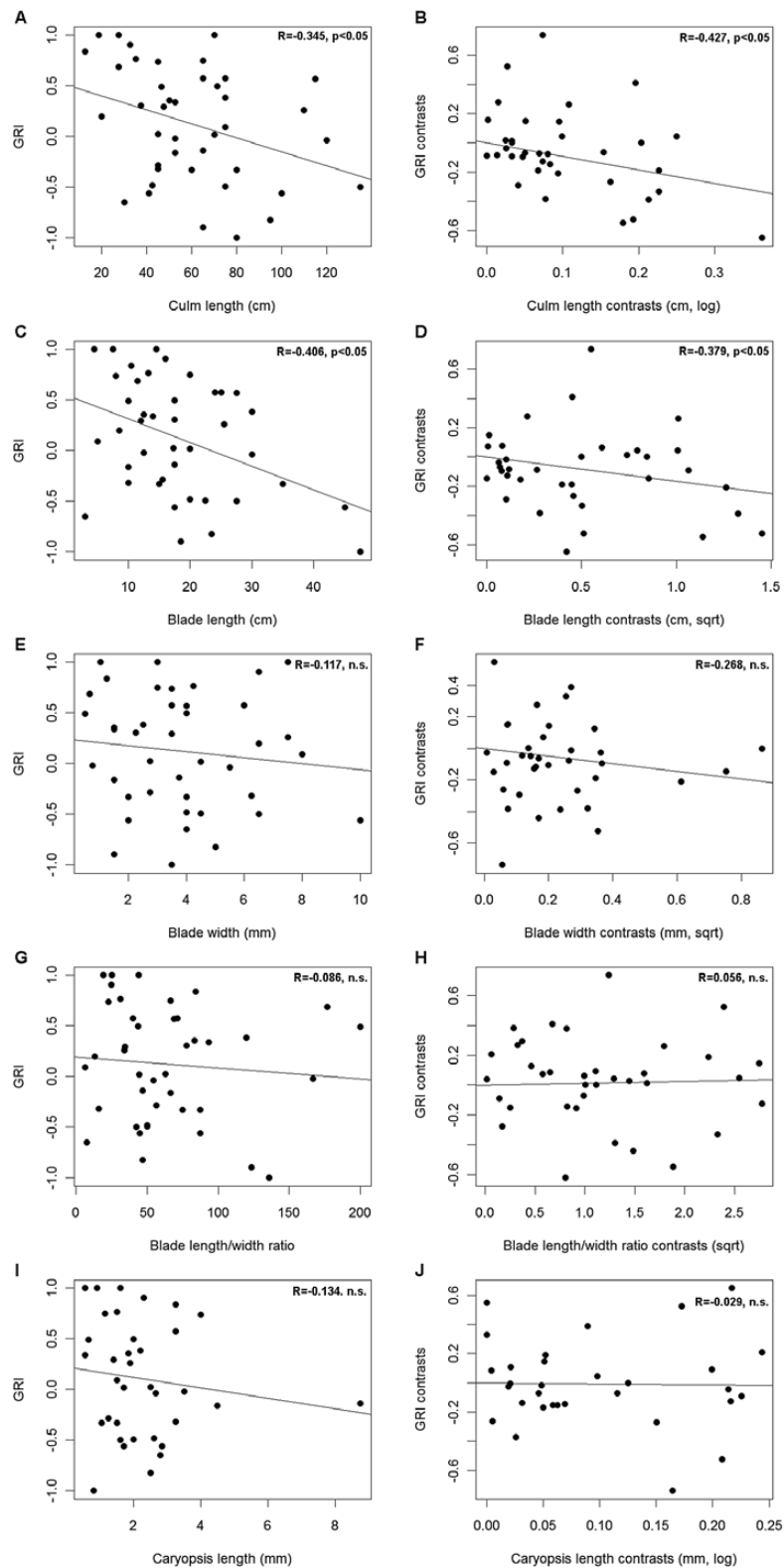
Figure 2: relationship between the GRI and 5 morphological traits of 41 Uruguayan grasses: culm length (**A**, **B**), blade length (**C**, **D**), blade width (**E**, **F**), blade length/width ratio (**G**, **H**) and caryopsis length (**I**, **J**). Left-hand panels show correlations based on the species trait values and the right-hand panels show the corresponding patterns for phylogenetic independent contrasts. Each point (i.e.contrast) represents the difference in the corresponding trait values between two sister taxa and reflects an independent evolutionary divergence. Correlation analyses using independent contrasts were calculated through the origin.

predictions for subhumid grasslands with a long history of grazing in the generalized model of Milchunas *et al.* (1988).

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