

# Does grazing induce intraspecific trait variation in plants from a sub-humid mountain ecosystem?

JUAN I. WHITWORTH-HULSE,<sup>1\*</sup> ANA M. CINGOLANI,<sup>1,2</sup> SEBASTIÁN R. ZEBALLOS,<sup>1,2</sup> MARÍA POCA<sup>1,2</sup> AND DIEGO E. GURVICH<sup>1,2</sup>

<sup>1</sup>*Instituto Multidisciplinario de Biología Vegetal (CONICET – Universidad Nacional de Córdoba), Córdoba, Argentina (Email: jiwwhulse@gmail.com) and* <sup>2</sup>*Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, Córdoba, Argentina*

**Abstract** Livestock grazing represents an important human disturbance for vegetation worldwide. We analysed the intraspecific differences in mean trait values between different grazing regimes (ungrazed and grazed) and explored whether these differences are consistent across species in a sub-humid mountain ecosystem in Central Argentina. We selected 14 species of eight different families, co-occurring in both regimes and comprising herbaceous (grasses and forbs) and woody (shrubs and trees) plants. For each species and grazing regime we measured 12 traits related to plant size, carbon fixation and water use. We found that plants in the grazed regime had consistently smaller leaves and shorter stature and internodal length than plants of the same species under the ungrazed regime. For the remaining traits the responses were species-specific. Dry matter content, leaf tensile strength and minimum leaf water potential ( $\Psi_{\text{leaf}}$ ) showed contrasting responses to grazing. Specific leaf area, wood density and potential water content of wood showed almost no significant responses except for very few species. Neither leaf area per shoot mass nor leaf area per sapwood area differed significantly between grazing regimes. Our study suggested that the intraspecific variation found for the size-related traits would allow species to respond to grazing without modifying markedly other structural traits, a plastic response that might increase the probability of species success.

**Key words:** domestic livestock, functional ecology, grazing response, intraspecific trait variation, sub-humid ecosystem.

## INTRODUCTION

Grazing by large herbivores is an important disturbance for vegetation and plays a central role in maintaining ecosystem structure and function (McNaughton 1979; Huntly 1991). Domestic livestock raising is one of the most important land uses in mountain ecosystems (Eckholm 1975; Kohler *et al.* 2006). The analysis of plant responses to herbivory has been largely based on plant functional traits because, besides their ecological significance, they are easy to measure (Díaz *et al.* 2001; Garnier *et al.* 2007). Trait-based approaches may improve our understanding not only of plant response to grazing, but also of the mechanisms underlying vegetation changes in grasslands and woodlands (Vesk & Westoby 2001; Lavorel & Garnier 2002; Díaz *et al.* 2007).

Global-scale studies have shown increases in short and prostrate plants with grazing (Díaz *et al.* 2001, 2007; Lavorel *et al.* 2011). In addition, grazing has been found to favour annual over perennial species, as well as stoloniferous and rosette architecture over

tussock architecture (Díaz *et al.* 2007). Other studies at the interspecific level reported that in systems with high availability of soil resources, grazing disturbance favours more acquisitive species (*sensu* Díaz *et al.* 2001; Reich 2014), which are those with high specific leaf area and low leaf dry matter content, which promote fast carbon acquisition and use (Díaz *et al.* 2001; Wright *et al.* 2004; Castro *et al.* 2010). For instance, in sub-humid grasslands, grazing was found to favour small species with high specific leaf area and low leaf tensile strength (Díaz *et al.* 2001; McIntyre & Lavorel 2001; Cingolani *et al.* 2005).

In contrast with the high number of studies addressing herbivore effects on plants at the interspecific level, the variation within species has been far less studied (*but see* Oosterheld & McNaughton 1988; Loreti *et al.* 2001). However, intraspecific trait variation studies have increased in the last decades highlighting the importance of addressing variation at this level (Bolnick *et al.* 2011; Violle *et al.* 2012; Siefert *et al.* 2015). Studies within species have revealed that grazing triggers changes in plant morphology, mainly in grass species (McNaughton 1984; Díaz *et al.* 1992). Grasses undergo a decrease in their stature and become more prostrate as grazing pressure increases (Kotanen & Bergelson 2000; McIntyre & Lavorel 2001). Additionally, an increase of specific leaf

\*Corresponding author.

Accepted for publication 1 February 2016.

area in response to grazing has been reported for *Poa ligularis* (Rotundo & Aguiar 2008). In some cases, plants under heavy grazing pressure also showed an increase of leaf water potential (i.e. less negative values) and leaf conductance to water vapour compared with individuals of the same species living in ungrazed or lightly grazed sites (Svejar & Christiansen 1987; Day & Detling 1994). It is important to recognize that intraspecific variability could be the result of phenotypic plasticity and genetic differentiation among populations, two mechanisms that are not mutually exclusive (Schlichting 1986; Tomás *et al.* 2000; Valladares *et al.* 2007).

In the sub-humid mountain ecosystem of Central Argentina, the patterns of intraspecific trait variation in response to grazing have been poorly studied. Previous studies by Díaz *et al.* (1992, 1994) have shown that grazing reduces plant size and leaf area of herbaceous species. However, the intraspecific response to grazing of other traits related to either carbon fixation (e.g. specific leaf area) or water use (e.g. minimum leaf water potential) remains unknown for species of this or other sub-humid mountain ecosystems. In the present study, we focused on trait variation among individuals of the same species in relation to grazing in Córdoba Mountains, Central Argentina. We did not distinguish genetic from phenotypic effects; thus, we refer to that variation as intraspecific trait variation (*sensu* Albert *et al.* 2010). We are particularly interested in elucidating the trait or traits that are most responsive to grazing and the magnitude and direction of the grazing effect. Accordingly, we addressed the following questions: (i) what are the intraspecific differences in mean trait values between grazed and ungrazed sites for a set of representative species?; and (ii) are these differences consistent across species?

## MATERIALS AND METHODS

### Study area

The study was conducted in the Quebrada del Condorito National Park and surrounding privately owned areas, located in Pampa de Achala (31° 37' S, 64° 42' W), a sub-humid ecosystem in the upper belt of Córdoba mountains, Central Argentina (1900–2100 m a.s.l.). Mean temperature of the coldest and warmest months are 5.0 °C and 11.4 °C, respectively, and there is no frost-free period (Cabido 1985). Mean annual precipitation is 889 mm, concentrated in the warm months, from October to April (Cabido 1985; Colladon 2014). The landscape consists of a mosaic of grasslands, *Polylepis australis* woodlands, granite outcrops and eroded areas with exposed rock surfaces (Cabido 1985; Cingolani *et al.* 2004). Small wild herbivores such as micro-mammals (e.g. rodents) occur in the study area, while large wild herbivores (mainly *Lama guanicoe*) were replaced by domestic livestock after Spaniards arrived at the beginning of the 17<sup>th</sup> century (Díaz *et al.* 1994; Cingolani *et al.* 2014). In 2007 *L. guanicoe* began to be reintroduced in the

National Park, but populations are yet small and the home ranges of the few groups are located away from our sampling area (Flores *et al.* 2013). Nowadays, cattle production is the main economic activity in Pampa de Achala highlands (Cingolani *et al.* 2003).

### Species selection and field sampling

We performed our study during 2010 and 2011 growth seasons, between late January and early March. During these years, annual precipitation was lower than the long-term average, summing 799 and 730 mm for 2009–2010 and 2010–2011 hydrologic years, respectively (Cingolani *et al.* 2015). We performed our study selecting individual plants of 14 native species from sites with contrasting grazing regimes (i.e. grazed and adjacent ungrazed sites). Based on previous studies (Díaz *et al.* 2001; Cingolani *et al.* 2003; Giorgis *et al.* 2010), we selected the most frequent species co-occurring in both regimes, although not necessarily in the same abundance, comprising herbaceous (grasses and forbs) and woody (shrubs and trees) plants (see Table 1 for the complete list of species).

The field sampling involved two areas (approximately 50 ha of land each) separated among them by about 8 km (hereafter ‘eastern’ and ‘western’ areas), because not all plant species assessed in this study occur in a single area. Each area was at the limit between an ungrazed and a grazed paddock separated

**Table 1.** Complete name, abbreviation and family of the 14 plant species

Species	Abbreviation	Family
Herbaceous		
<i>Carex fuscua</i> d’Urv. ssp. <i>fuscua</i>	Car fus	Cyperaceae
<i>Deyeuxia hieronymi</i> (Hack.) Türpe	Dey Hie	Poaceae
<i>Eryngium agavifolium</i> Griseb.	Ery aga	Apiaceae
<i>Eryngium nudicaule</i> Lam.	Ery nud	Apiaceae
<i>Festuca lilloi</i> Hack.	Fes lil	Poaceae
<i>Juncus uruguensis</i> Griseb.	Jun uru	Juncaceae
<i>Lachemilla pinnata</i> (Ruiz & Pav.) Rothm.	Lac pin	Rosaceae
<i>Muhlenbergia peruviana</i> (P. Beauv.) Steud	Muh per	Poaceae
<i>Poa stuckertii</i> (Hack.) Parodi	Poa stu	Poaceae
Woody		
<i>Baccharis tucumanensis</i> Hook. & Arn. var. <i>tucumanensis</i>	Bac tuc	Asteraceae
<i>Berberis hieronymi</i> C.K. Schneid	Ber hie	Berberidaceae
<i>Clinopodium odorum</i> (Griseb.) Harley	Cli odo	Lamiaceae
<i>Polylepis australis</i> Bitter	Pol aus	Rosaceae
<i>Stevia achalensis</i> Hieron.	Ste ach	Asteraceae

by wire fences. In the eastern area, the ungrazed paddock was located within the Quebrada del Condorito National Park, in a sector without livestock since 1997, whereas the grazed paddock was part of a privately owned property. In the western area, both the grazed and ungrazed paddocks were entirely within the National Park. In this case, the ungrazed paddock was a small enclosure from the year 2003. In the eastern area we collected individual plants of 12 out of the 14 species, and in the western area we collected the remaining two selected species, *P. australis* and *Berberis hieronymi* (Cingolani *et al.* 2003). We indirectly estimated the localized stocking rate of each grazed paddock measuring the frequency of livestock dung (following von Müller *et al.* 2012), and we obtained values of approximately 0.8 cattle units/ha and 0.6 cattle units/ha for the eastern and western areas respectively.

At each grazing regime, we randomly collected five to 10 individual plants per species that were at least 20 m apart. Sampled plants were sexually mature, healthy, unshaded and without any evidence of damage by pathogens or herbivores (following Pérez-Harguindeguy *et al.* 2013). The grazed sampling paddocks were stocked at the time of the study, but we ensured that the individual plants selected did not show direct immediate effects of defoliation. We gave particular attention to tussock species, and thus we verified that any tiller showed no traces of grazing.

### Plant trait measurements

Following standard methods (Pérez-Harguindeguy *et al.* 2013; Zeballos *et al.* 2014), we considered 12 functional traits related to plant size, carbon fixation and water use (Reich *et al.* 2003; Leishman *et al.* 2007; Reich 2014). We measured traits on five to 10 leaves and one to three twigs per individual plant for each species. Out of the 12 traits, five were measured for all species, five only for woody species and two only for herbaceous species. For all species we measured effective leaf area, specific leaf area (hereafter SLA), leaf dry matter content (hereafter LDMC), leaf tensile strength and minimum leaf water potential (hereafter  $\Psi_{\text{leaf}}$ ). For woody species only, we measured internodal length, wood density, potential water content of wood, leaf area per shoot mass and leaf area per sapwood area (inverse of Huber value). Finally, for herbaceous species only, we measured plant height and total leaf area.

We measured effective leaf area ( $\text{mm}^2$ ; Ackerly 2004; Zeballos *et al.* 2014) on 10 fresh leaves per individual by scanning them and estimated the area using the software Leaf Area© (University of Sheffield, UK). For species with compound leaves, we considered individual leaflets. We then oven-dried the samples at 70 °C for 72 h, and weighed them to calculate SLA ( $\text{mm}^2 \text{mg}^{-1}$ ; Pérez-Harguindeguy *et al.* 2013). We calculated LDMC ( $\text{mg g}^{-1}$ ) as leaf dry mass divided by its water-saturated fresh mass (Vaieretti *et al.* 2007). For leaf tensile strength ( $\text{N mg}^{-1}$ ) measurements, we used a tensile-strength meter constructed on the basis of Hendry and Grime (1993), and expressed it as force needed per unit of width of a leaf sample. We measured  $\Psi_{\text{leaf}}$  (MPa) with a pressure chamber

(Scholander *et al.* 1965). We performed these measurements at midday on sunny days, between 12:30 and 13:00 h (Zeballos *et al.* 2014).

We measured internodal length (mm) with a caliper on three stems per individual woody plant species (Stobbs 1973). We determined wood density ( $\text{mg mm}^{-3}$ ) as the dry mass divided by volume (Jacobsen *et al.* 2008). We soaked the stem segments in water for 24 h, and then calculated their volumes with Archimedes' principle using a graduated test tube (10:0.1 mL). We obtained dry mass by oven-drying the samples at 70 °C for 72 h and weighing them. We calculated the potential water content of wood (%) as: [(saturation mass – dry mass) per dry mass]  $\times$  100 (Borchert 1994). In this case, we used the same samples employed for wood density. For both leaf area per shoot mass ( $\text{mm}^2 \text{g}^{-1}$ ) and leaf area per sapwood area ( $\text{mm}^2 \text{mm}^{-2}$ ), we calculated the leaf area of a terminal branch by multiplying total leaf dry mass per SLA (Wright *et al.* 2006). We previously separated the leaves from the stems of the terminal branch, oven-dried the samples at 70 °C for 72 h and weighed them to obtain dry mass of leaves and stem, respectively. On the one hand, we calculated shoot mass as: (leaf dry mass + stem dry mass), and on the other, we determined sapwood cross-sectional area by measuring the diameter of the twig from where it was cut with a caliper after the bark had been removed.

We measured plant height (cm) of herbaceous species as the distance between the top of the main photosynthetic tissues on a plant and the ground level (Pérez-Harguindeguy *et al.* 2013). We also calculated total leaf area ( $\text{mm}^2$ ) using the species means of effective leaf area and multiplied these values by the number of mature photosynthetic active leaves.

### Data analyses

We analysed data at two hierarchical levels: within and across species. In the first level, we analysed in detail how each individual species responded to grazing; and in the second, we analysed the consistency of the trait response to grazing across species.

At the within-species level, for all species we compared mean values of each functional trait between ungrazed and grazed regimes using *t*-tests. In this analysis, each species was analysed independently of the others, using individuals as replicates and data in all cases met the assumptions of normality. For the sake of a better visual comparison among results of traits with different measurement units, we calculated for each species the proportional difference of each mean trait value between ungrazed and grazed regimes as a percentage (difference (%) = (grazed – ungrazed)  $\times$  100 / ungrazed), and we included these percentages in the report of the results.

At the across-species level, we examined for each trait whether the response to grazing was consistent across species. For this analysis, we considered each species as a case, and used the average of each trait for each grazing regime ( $n = 14$  species for effective leaf area, SLA, LDMC, leaf tensile strength and  $\Psi_{\text{leaf}}$ ;  $n = 5$  species for internodal length, wood density,

potential water content of wood, leaf area per shoot mass, leaf area per sapwood area;  $n = 9$  species for plant height and total leaf area). For each trait, we performed paired comparisons (paired  $t$ -tests) between the ungrazed and the grazed regimes. In this case, we Ln transformed effective leaf area and total leaf area to attain normality.

Results of analyses at both levels were considered statistically significant when  $P < 0.05$ . All analyses were carried out in R 2.15.1 (R Development Core Team 2012), using the MASS package.

## RESULTS

### Within-species analyses

All species showed significant variation in response to grazing in at least one trait, but none showed differences in all of the measured traits (Table 2). Ten out of the 12 traits varied significantly for at least one species, while two did not vary for any species.

Out of the five traits measured for all species, effective leaf area showed the highest number of significant responses. It was significantly reduced in response to grazing for nine out of the 14 species (Table 2a), whereas no species showed a significant increase. For those nine species, the reduction varied from 28% (760.8 to 546.7 mm<sup>2</sup>) in the case of *P. australis* to 68% (10 483.3 to 3395.7 mm<sup>2</sup>) in the case of *Eryngium agavifolium* (Fig. 1a). In contrast, for the remaining traits measured for all species (i.e. SLA, LDMC,  $\Psi_{\text{leaf}}$  and leaf tensile strength), the responses were significant only in a few cases (Table 2a; Fig. 1b–e). Significant differences only involved three species in SLA and LDMC, four in  $\Psi_{\text{leaf}}$  and six out of 14 species in leaf tensile strength. For the three species with a significant SLA variation, we found a decrease in their mean values which varied from 32% (11.4 to 7.8 mm<sup>2</sup> mg<sup>-1</sup>) in the case of *E. agavifolium* to 61% (13.5 to 5.2 mm<sup>2</sup> mg<sup>-1</sup>) in the case of *Festuca lilloi* (Fig. 1b). Species varying significantly in LDMC, leaf tensile strength and  $\Psi_{\text{leaf}}$  showed contrasting responses (Fig. 1c–e). For example, while the mean LDMC value of *Muhlenbergia peruviana* and *Juncus uruguensis* decreased 34% (275.8 to 182.4 mg g<sup>-1</sup>) and 8% (365. to 337.4 mg g<sup>-1</sup>) respectively between ungrazed and grazed regimes, *Poa stuckerti* increased 19% (344.6 to 409.5 mg g<sup>-1</sup>) (Fig. 1c).

Regarding traits measured only in woody species (Fig. 1f–j), internodal length was significantly reduced in response to grazing for all species (Table 2b). Grazing resulted in shorter stem internodes, varying from 41% (19.6 to 11.53 mm) in the case of *B. hieronymi* to 62% (37 to 14.07 mm) in the case of *Clinopodium odorum* (Fig. 1f). In turn, for wood density and potential water content of wood, only one out of the five species varied significantly in each trait, *Baccharis tucumanensis* and

*Stevia achalensis* respectively (Table 2b). In particular, wood density showed an important increase of 142% (0.31 to 0.75 mg mm<sup>-3</sup>) for *B. tucumanensis* (Fig. 1g), while potential water content of wood showed a decrease of 33% (165.5 to 110.8%) for *S. achalensis* (Fig. 1h). Finally we found that neither leaf area per shoot mass nor leaf area per sapwood area differed significantly between grazing regimes for any species (Table 2; Fig. 1i–j).

Considering the traits measured only in herbaceous species, we found that plant height was significantly reduced in response to grazing for all species (Table 2c). Grazing generated shorter individual plants and the height reduction varied from 31% (90.8 to 63 cm) for *Deyeuxia hieronymi* to 90% (59.8 to 5.4 cm) for *E. agavifolium* (Fig. 1k). In the case of total leaf area we found significant variation for seven out of the nine species measured, showing a decrease in its mean values under the grazed regime (Table 2c). The reduction was very variable among species, reaching values as high as 83% for *J. uruguensis* (Fig. 1l).

### Across-species analyses

We found a significantly consistent decrease in effective leaf area with grazing across all species (Table 3). In contrast, the remaining traits measured for all species (SLA, LDMC,  $\Psi_{\text{leaf}}$  and leaf tensile strength) did not vary consistently with grazing (Table 3). We also found that the decrease in internodal length with grazing was significantly consistent across all woody species, whereas the response of wood density, potential water content of wood, leaf area per shoot mass and leaf area per sapwood area (i.e. the remaining traits measured for woody species only) was not consistent (Table 3). Finally, we found that plant height and total leaf area decreased consistently with grazing across herbaceous species (Table 3).

## DISCUSSION

In this study we used a trait-based approach to examine plant trait variations in response to livestock grazing at the intraspecific level. Size-related traits (i.e. effective leaf area, internodal length, plant height and total leaf area) showed a consistent reduction in response to grazing across species. Nonetheless, results for the remaining eight traits reflecting carbon fixation and water use showed a weak and/or inconsistent response to grazing pressure across species.

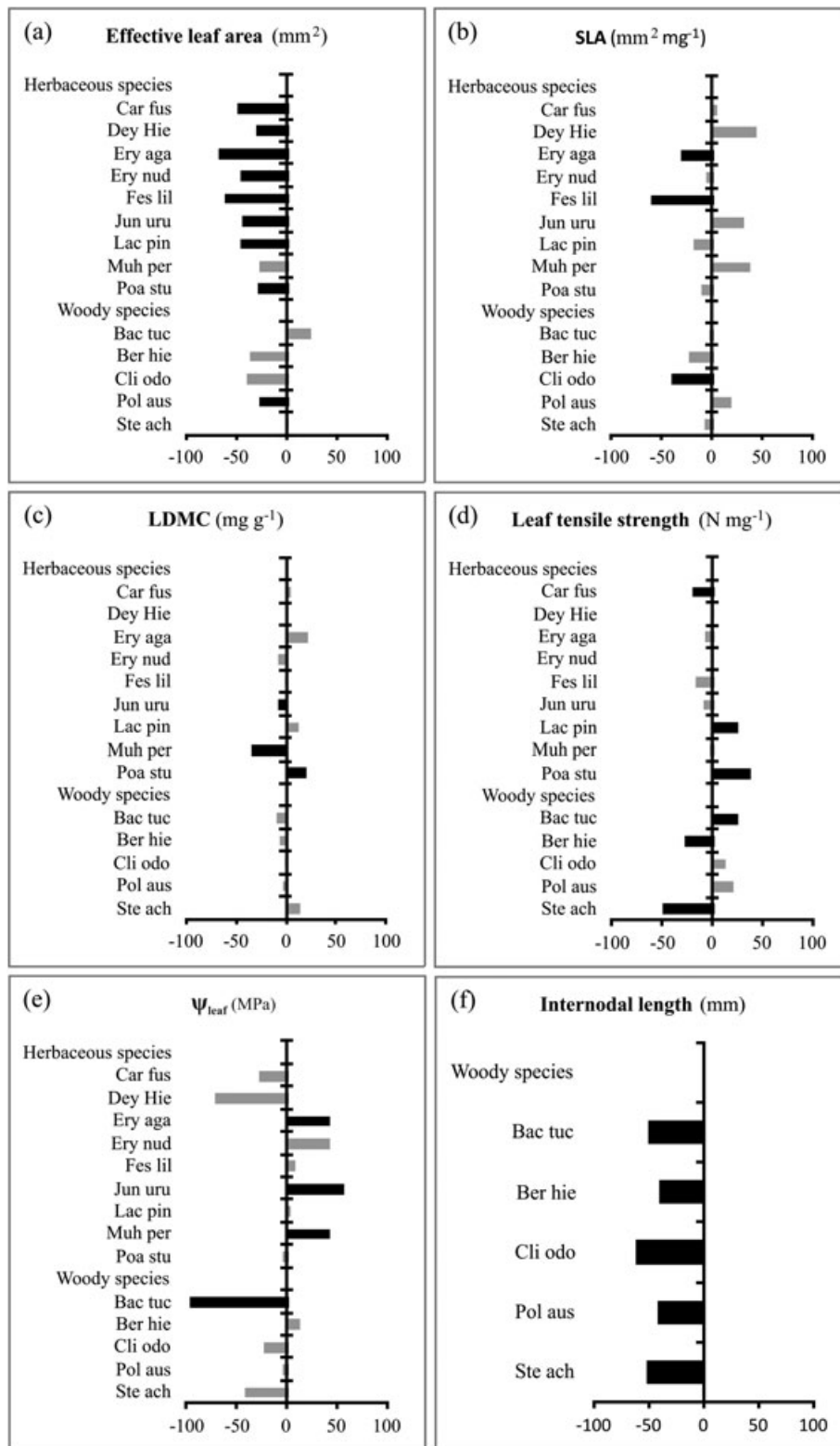
The clear trend of variation of effective leaf area, internodal length, plant height and total leaf area showed that individual plants growing under a grazed regime had smaller leaves, shorter internodes and also shorter stature than plants of the same species growing in an

**Table 2.** Within-species comparisons in traits measured for all species (a), woody species only (b) and herbaceous species only (c). Mean trait values of each species in ungrazed (UnG) and grazed regimes (G) are shown. For each species significant differences ( $P < 0.05$ ) between grazing regimes are indicated in bold letters. Individual replicates per species at each grazing regime are in parentheses

a) Traits	Effective leaf area (mm <sup>2</sup> ) (n = 10)		SLA (mm <sup>2</sup> mg <sup>-1</sup> ) (n = 5)		LDMC (mg g <sup>-1</sup> ) (n = 5)		Leaf tensile strength (N mg <sup>-1</sup> ) (n = 5)		$\Psi_{\text{leaf}}$ (MPa) (n = 5)	
	UnG	G	UnG	G	UnG	G	UnG	G	UnG	G
Herbaceous										
<i>Carex fuscua</i>	575.2	<b>294.7</b>	15.2	15.8	312.2	322.2	2.9	<b>2.3</b>	-3.7	-4.8
<i>Deyeuxia hieronymi</i>	401.3	<b>276.8</b>	11.2	15.9	368	370	7.6	7.7	-2.4	-4
<i>Eryngium agavifolium</i>	10 483.3	<b>3395.7</b>	11.4	<b>7.8</b>	150.6	180.3	3.2	3	-2.7	<b>-1.6</b>
<i>Eryngium nudicaule</i>	788.6	<b>429.0</b>	15.2	14.2	201.6	186.1	1.1	1.1	-2.1	-1.3
<i>Festuca lilloi</i>	481.4	<b>184.1</b>	13.5	<b>5.2</b>	478.8	472.7	36.4	30	-4.4	-4.1
<i>Juncus uruguensis</i>	235.2	<b>128.7</b>	4.6	6.0	365.1	<b>337.4</b>	24.4	22.1	-5.2	<b>-2.4</b>
<i>Lachemilla pinnata</i>	683.3	<b>363.8</b>	26.7	21.7	238.5	266.1	0.4	<b>0.5</b>	-3.4	-3.3
<i>Muhlenbergia peruviana</i>	28.3	20.5	16.2	22.2	275.8	<b>182.4</b>	1.5	1.4	-7.1	<b>-4.2</b>
<i>Poa stuckertii</i>	7058.4	<b>5017.6</b>	9.8	8.8	344.6	<b>409.6</b>	17.1	<b>23.2</b>	-2.8	-2.9
Woody										
<i>Baccharis tucumanensis</i>	217.4	265.9	10	9.8	245.5	221.5	0.8	<b>1.0</b>	-1.5	<b>-3</b>
<i>Berberis hieronymi</i>	65.9	41.7	6.8	5.2	447.2	416.3	1.6	<b>1.2</b>	-3.3	-2.9
<i>Clinopodium odorum</i>	156.6	93.7	21	<b>12.7</b>	250.6	249.8	0.6	0.7	-2	-2.5
<i>Polylepis australis</i>	760.8	<b>546.7</b>	9.1	10.7	374.5	362.7	0.8	1	-2	-2
<i>Stevia achalensis</i>	162.5	165.1	14.6	13.5	241	271.37	0.8	<b>0.4</b>	-1.7	-2.4
SLA, specific leaf area; LDMC, leaf dry matter content; $\Psi_{\text{leaf}}$ minimum leaf water potential										
b) Traits	Internodal length (mm) (n = 5)		Wood density (mg mm <sup>-3</sup> ) (n = 5)		Potential water content of wood (%) (n = 5)		Leaf area per shoot mass (mm <sup>2</sup> g <sup>-1</sup> ) (n = 5)		Leaf area per sapwood area (mm <sup>2</sup> mm <sup>-2</sup> ) (n = 5)	
	UnG	G	UnG	G	UnG	G	UnG	G	UnG	G
Woody species										
<i>Baccharis tucumanensis</i>	35.9	<b>18</b>	0.3	<b>0.8</b>	128.9	114.1	2746.3	3780.7	1598	2654.5
<i>Berberis hieronymi</i>	19.6	<b>11.5</b>	0.7	0.6	81.9	86.5	2546.7	1746	2368	1365
<i>Clinopodium odorum</i>	37	<b>14.1</b>	0.5	0.4	117.2	127.6	7521.9	5324.6	2695.5	2263.7
<i>Polylepis australis</i>	61.2	<b>35.4</b>	0.5	0.6	117.6	106.7	2921	3445.3	2106.1	1706.6
<i>Stevia achalensis</i>	37.4	<b>18.1</b>	0.3	0.5	165.5	<b>110.8</b>	8121.9	7029.2	2855.1	2918.2
c) Traits										
Species	Plant height (cm) (n = 10)				Total leaf area (mm <sup>2</sup> ) (n = 5)					
	UnG		G		UnG		G			
Herbaceous species										
<i>Carex fuscua</i>	18.6		<b>5.8</b>		8081		<b>3000.7</b>			
<i>Deyeuxia hieronymi</i>	90.8		<b>63</b>		767 714.4		<b>295 807.7</b>			
<i>Eryngium agavifolium</i>	59.8		<b>5.4</b>		138 030.2		<b>49 487.5</b>			
<i>Eryngium nudicaule</i>	6		<b>1.3</b>		45 551.8		26 044.6			
<i>Festuca lilloi</i>	114		<b>93</b>		395 727.4		<b>126 697.6</b>			
<i>Juncus uruguensis</i>	31		<b>12.2</b>		5202		<b>885.5</b>			
<i>Lachemilla pinnata</i>	16.1		<b>1.7</b>		5399.5		<b>2052.9</b>			
<i>Muhlenbergia peruviana</i>	9.4		<b>3.1</b>		4666.8		2706.8			
<i>Poa stuckertii</i>	141		<b>93.8</b>		6 226 216.9		<b>2 766 834.8</b>			

ungrazed place. Our results were in accordance with previous studies in sub-humid mountain ecosystems (Díaz *et al.* 1992; McIntyre & Lavorel 2001; Giorgis *et al.* 2010), as well as with trends documented for other regions in the world (Reisch & Poschlod 2003; Dorrrough *et al.* 2004; Gross *et al.* 2007). It has been suggested that the miniaturization of leaves and the concentration of biomass close to the ground may reduce the ability of grazing animals to detect plants and also limit the prehensile abilities of livestock (Stobbs 1973; Chacon &

Stobbs 1976), leading to lower levels of damage on leaves and flowers (Westoby 1980; Díaz *et al.* 1992). In the case of herbaceous plants, other reason for their stature reduction can be that the lack of light competition under grazed regime may favour plants with a smaller canopy (Kotanen & Bergelson 2000). Tomás *et al.* (2000), analysing the herbaceous *Piptochaetium napostaense*, found that morphological differentiation among plants under different grazing regimes (i.e. enclosure and grazed sites), especially height reduction,



**Fig. 1.** Direction and magnitude of plant trait variations for the 14 species measured in ungrazed and grazed regimes. (a–e) Traits measured for all species, (f–j) woody species only and (k–l) herbaceous species only. In all cases differences are expressed as percentage (%) relative to ungrazed values to facilitate comparison of the results for the different traits. Difference (%) =  $(\text{grazed} - \text{ungrazed}) \times 100 / \text{ungrazed}$ . Black bars indicate significant variations ( $P < 0.05$ ) in mean value of each trait between grazing regimes. Abbreviation, the corresponding complete name and family of each species are shown in Table 1.

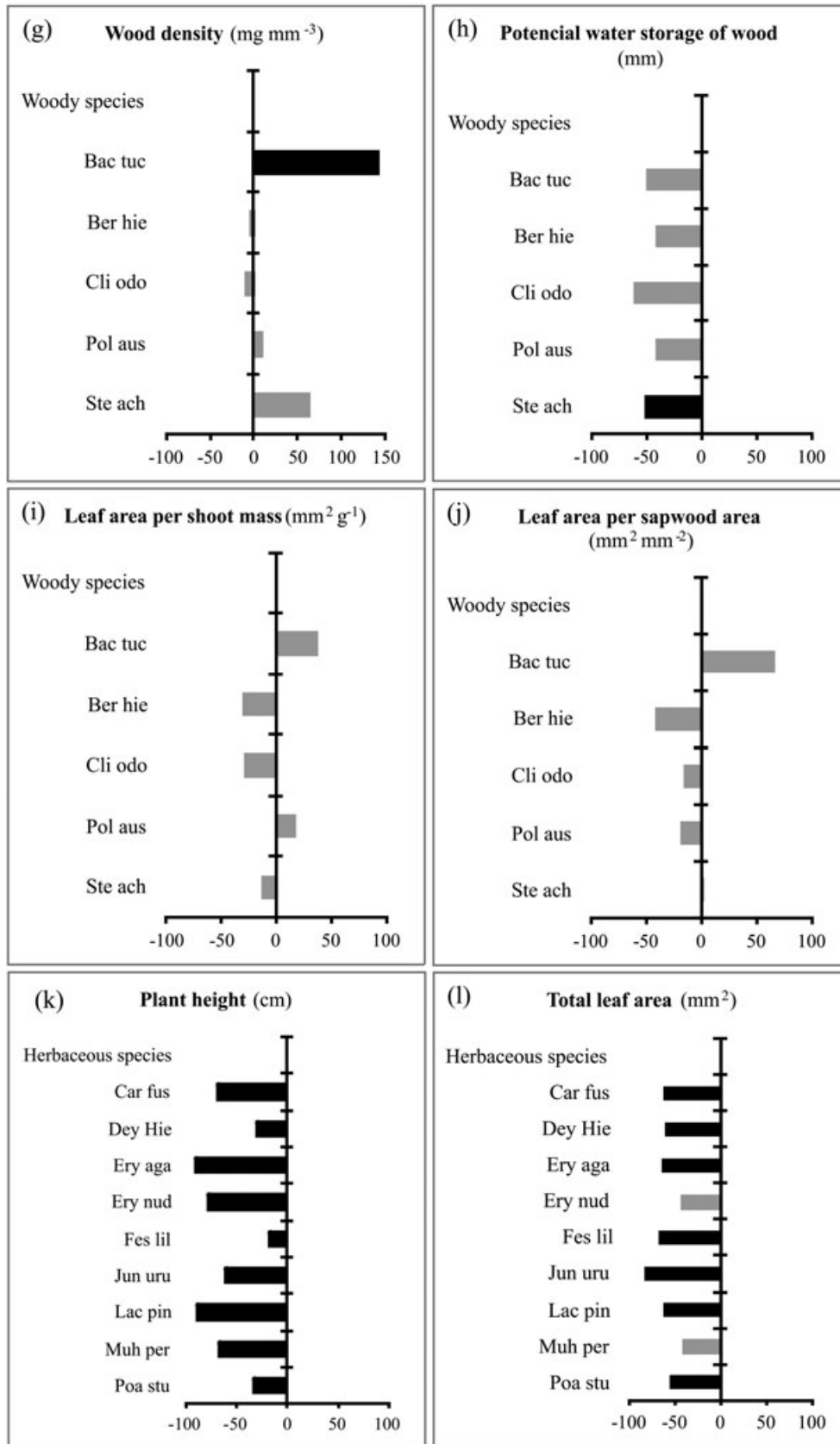


Fig. 1. (Continued)

**Table 3.** Across-species comparisons (paired *t*-tests) between the ungrazed and grazed regimes for plant traits measured for all species, woody species only and herbaceous species only. Significant paired differences are indicated in bold letters ( $P < 0.05$ )

Traits	Mean difference (grazed - ungrazed)	<i>P</i>
All species ( $n = 14$ ) <sup>†</sup>		
Effective leaf area (Ln) (mm <sup>2</sup> )	-0.21	< <b>0.001</b>
SLA (mm <sup>2</sup> mg <sup>-1</sup> )	-1.13	0.332
LDMC (mg g <sup>-1</sup> )	-3.25	0.750
Leaf tensile strength (N mg <sup>-1</sup> )	-0.26	0.712
$\Psi_{leaf}$ (MPa)	0.2	0.590
Woody species ( $n = 5$ ) <sup>†</sup>		
Internodal length (mm)	-18.08	<b>0.003</b>
Wood density (mg mm <sup>-3</sup> )	0.12	0.261
Potential water content of wood (%)	-13.09	0.316
Leaf area per shoot mass (mm <sup>2</sup> g <sup>-1</sup> )	-506.4	0.432
Leaf area per sapwood area (mm <sup>2</sup> mm <sup>2</sup> )	-142.94	0.699
Herbaceous species ( $n = 9$ ) <sup>†</sup>		
Plant height (cm)	-23.04	<b>0.004</b>
Total leaf area (Ln) (mm <sup>2</sup> )	-0.42	< <b>0.001</b>

<sup>†</sup>We considered each species as a case, and used the average of each trait for each grazing regime

appears to be the result of a phenotypically plastic response of adapted genotypes. Accordingly, although our study was not designed to differentiate local adaptation and phenotypic plasticity, we found differences in the internodal length of woody species, a morphological variation that has been reported as a plastic response to herbivory (Gianoli *et al.* 2007).

In sub-humid ecosystems, interspecific studies addressing the response to grazing of traits related to carbon fixation showed that species with low SLA and high LDMC and leaf tensile strength are replaced by species with the opposite trait attributes (Díaz *et al.* 2001; Cingolani *et al.* 2005). The latter species, with high SLA and low LDMC, may have high growth rate and invest less resources in structural protection of the photosynthetic tissues, as an adaptation to tolerate the effect of defoliation (Díaz *et al.* 2001; Cornelissen *et al.* 2003; Cingolani *et al.* 2005). Conversely, our results within species were not consistent with those well-documented findings between species and in turn showed species-specific responses to grazing. In the case of SLA, LDMC, leaf tensile strength and leaf area per shoot mass, grazing either leads to a complex set of idiosyncratic intraspecific variations, exhibiting responses in different directions, or has no significant effects at all. For instance, our study revealed that SLA decreased or did not vary significantly with grazing, whereas the studies at the interspecific or community level in the same ecosystem showed an increase of this trait (Díaz *et al.* 2001; Cingolani *et al.* 2007, 2014). Following the latter trend, Rotundo and Aguiar (2008), examining trait

variations in the Patagonian arid steppe of South America, found plants of *P. ligularis* with higher SLA under a grazed regime than an ungrazed one, a result that was also inconsistent with our findings. Actually, in our study the responses at intraspecific level of traits reflecting carbon fixation could be an effect of either the closeness or openness of the vegetation rather than a response to grazing, or even both light and grazing effects may be interacting. Particularly, SLA variation could be explained by the dominance of tussock species in the ungrazed regime (Cingolani *et al.* 2014), which may reduce light availability and consequently cause a SLA increase of the small species that live below the canopy of tussock grasses (Rusch *et al.* 2009; Cingolani *et al.* 2014).

Species-specific responses to grazing emerge not only in traits that are associated with carbon fixation (e.g. SLA, LDMC and leaf tensile strength), but also in other traits related to water use, such as  $\Psi_{leaf}$ , wood density and potential water content of wood. For example, many studies analysing  $\Psi_{leaf}$  variation in response to grazing (Archer & Detling 1986; Kalapos 1994; Harrison *et al.* 2010) have reported positive effects of grazing on the water status of species (i.e. less negative  $\Psi_{leaf}$  values under grazing conditions). It has been suggested that the intraspecific reduction of the plant transpirational surface in response to grazing may reduce water consumption and enhance soil moisture in grazed sites, improving the water status of those plants (Svejar & Christiansen 1987; Bremer *et al.* 2001; Wang *et al.* 2012). Whilst some of our selected species increased mean  $\Psi_{leaf}$  in response to grazing in agreement with this general trend, *B. tucumanensis* showed a decrease of  $\Psi_{leaf}$  and other species did not respond to grazing, as previously found in other studies (Nowak & Caldwell 1984; Pittermann *et al.* 2014). Therefore, the absence of patterns for traits reflecting water use also suggests that species are affected by grazing in idiosyncratic ways.

Our study suggested that the intraspecific variation found for the size-related traits would allow species to respond to grazing without modifying markedly other structural functional traits, a plastic response that might increase the probability of species success in the sub-humid mountain ecosystem of Central Argentina (Chesson 2000; Tomás *et al.* 2000; Shipley *et al.* 2006). Nonetheless, more complex is the interpretation of the idiosyncratic intraspecific response to grazing of those traits related to carbon fixation and water use. Following this trend, Albert *et al.* (2010) also reported idiosyncratic trait responses of species in the Central French Alps (e.g. for LDMC and leaf nitrogen content). However, that study was focused on climatic gradient variation (temperature and radiation) along the Alps. The authors attributed the idiosyncratic variation not only to climatic gradient *per se*, but also to other factors at local scales (e.g. soil properties). Therefore, these results suggest that other local factors, like micro-environmental



changes or even interactions among plants, could play an important role on intraspecific trait variation in the sub-humid mountain ecosystem of Central Argentina. As a consequence, we cannot discard that idiosyncratic responses could mean that species have different modes to respond to those factors (Del-Val & Crawley 2005). Accordingly, more detailed studies are needed to identify the idiosyncratic responses that we highlighted. For instance, future studies involving some fitness traits (e.g. relative growth rate, seed production) and also reciprocal transplant experiments could shed light on the lack of clear patterns found in the studied sub-humid mountain ecosystem, particularly for traits reflecting carbon fixation and water use.

## ACKNOWLEDGMENTS

We are grateful to CONICET (PIP 112-201201-00164 and 112-201101-00873) who founded this study, to the Quebrada del Condorito National Park personnel for logistic support and to C. Ferrero and T. Morán López who helped in the field work. J. Brasca, P. Amos and C. Whitworth-Hulse helped to improve English style. Authors are researchers and fellows of CONICET at Instituto Multidisciplinario de Biología Vegetal (CONICET-Universidad Nacional de Córdoba).

## REFERENCES

- Ackerly D. (2004) Functional strategies of chaparral shrubs in relation to seasonal water deficit and disturbance. *Ecol. Monogr.* **74** (1), 25–44.
- Albert C. H., Thuiller W., Yoccoz N. G., *et al.* (2010) Intraspecific functional variability: extent, structure and sources of variation. *J. Ecol.* **98**, 604–13.
- Archer S. & Detling J. K. (1986) Evaluation of potential herbivore mediation of plant water status in a North American mixed-grass prairie. *Oikos* **47**, 287–91.
- Bolnick D. I., Amarasekare P., Araújo M. S., *et al.* (2011) Why intraspecific trait variation matters in community ecology. *Trends Ecol. Evol.* **26**, 183–92.
- Borchert R. (1994) Soil and stem water storage determine phenology and distribution of tropical dry forest trees. *Ecology* **75**, 1437–49.
- Bremer D. J., Auen L. M., Ham J. M. & Owensby C. E. (2001) Evapotranspiration in a prairie ecosystem: effects of grazing by cattle. *Agron. J.* **93**, 338–48.
- Cabido M. (1985) Las comunidades vegetales de la pampa de Achala. Sierras de Córdoba. *Argent. Doc. Phytosociol.* **9**, 431–43.
- Castro H., Lehsten V., Lavorel S. & Freitas H. (2010) Functional response traits in relation to land use change in the Montado. *Agric. Ecosyst. Environ.* **137**, 183–91.
- Chacon E. & Stobbs T. H. (1976) Influence of progressive defoliation of a grass sward on the eating behavior of cattle. *Aust. J. Agric. Res.* **27**, 709–27.
- Chesson P. (2000) Mechanisms of maintenance of species diversity. *Annu. Rev. Ecol. Evol. Syst.* **31**, 343–66.
- Cingolani A. M., Cabido M. R., Renison D. & Solís N. V. (2003) Combined effects of environment and grazing on vegetation structure in Argentine granite grasslands. *J. Veg. Sci.* **14**, 223–32.
- Cingolani A. M., Renison D., Zak M. R. & Cabido M. R. (2004) Mapping vegetation in a heterogeneous mountain rangeland using landsat data: an alternative method to define and classify land-cover units. *Remote Sens. Environ.* **92**, 84–97.
- Cingolani A. M., Posse G. & Collantes M. B. (2005) Plant functional traits, herbivore selectivity and response to sheep grazing in Patagonian steppe grasslands. *J. Appl. Ecol.* **42**, 50–9.
- Cingolani A. M., Cabido M., Gurvich D. E., Renison D. & Díaz S. (2007) Filtering processes in the assembly of plant communities: are species presence and abundance driven by the same traits? *J. Veg. Sci.* **18**, 911–20.
- Cingolani A. M., Vaieretti M. V., Giorgis M. A., Poca M., Tecco P. A. & Gurvich D. E. (2014) Can livestock grazing maintain landscape diversity and stability in an ecosystem that evolved with wild herbivores? *Perspect. Plant Ecol. Evol. Syst.* **16**, 143–53.
- Cingolani A. M., Poca M., Giorgis M. A., *et al.* (2015) Water provisioning services in a seasonally dry subtropical mountain: identifying priority landscapes for conservation. *J. of Hydrol.* **525**, 178–87.
- Collado L. (2014) *Anuario Pluviométrico 1992–2012. Cuenca del Río San Antonio, Sistema del Río Suquia, Provincia de Córdoba.* Instituto Nacional del Agua y del Ambiente (INA) y Centro de Investigaciones de la Región Semiárida (CIRSA), Córdoba, Argentina.
- Cornelissen J. H. C., Garnier E., Lavorel S., *et al.* (2003) A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. *Aust. J. Bot.* **51**, 335–80.
- Day T. A. & Detling J. K. (1994) Water relations of *Agropyron smithii* and *Bouteloua gracilis* and community evapotranspiration following long-term grazing by prairie dogs. *Am. Midl. Nat. J.* **132**, 381–92.
- Del-Val E. & Crawley M. J. (2005) Are grazing increaser species better tolerators than decreasers? An experimental assessment of defoliation tolerance in eight British grassland species. *J. Ecol.* **93**, 1005–16.
- Díaz S., Acosta A. & Cabido M. (1992) Morphological analysis of herbaceous communities under different grazing regimes. *J. Veg. Sci.* **3**, 689–96.
- Díaz S., Acosta A. & Cabido M. (1994) Community structure in montane grasslands of central Argentina in relation to land use. *J. Veg. Sci.* **5**, 483–8.
- Díaz S., Lavorel S., McIntyre S., *et al.* (2007) Plant trait responses to grazing – a global synthesis. *Glob. Chang. Biol.* **13**, 313–41.
- Díaz S., Noy-meir I. & Cabido M. (2001) Can grazing response of herbaceous plants be predicted from simple vegetative traits? *J. Appl. Ecol.* **38**, 497–508.
- Dorrough J., Ash J. & McIntyre S. (2004) Plant responses to livestock grazing frequency in an Australian temperate grassland. *Ecography* **27**, 798–810.
- Eckholm E. P. (1975) The deterioration of mountain environments ecological stress in the highlands of Asia, Latin America, and Africa takes a mounting social toll. *Science* **189**, 764–70.
- Flores C. E., Cingolani A. M., von Müller A. & Barri F. R. (2013) Habitat selection by reintroduced guanacos (*Lama guanicoe*) in a heterogeneous mountain rangeland of central Argentina. *Rangeland J.* **34**, 439–45.
- Garnier E., Lavorel S., Ansquer P., *et al.* (2007) Assessing the effects of land-use change on plant traits, communities and ecosystem functioning in grasslands: a standardized methodology and lessons from an application to 11 European sites. *Ann. Bot.* **99**, 967–85.

- Gianoli E., Molina-Montenegro M. A. & Becerra J. (2007) Interactive effects of leaf damage, light intensity and support availability on chemical defenses and morphology of a twining vine. *J. Chem. Ecol.* **33**, 95–103.
- Giorgis M. A., Cingolani A. M., Teich I., Renison D. & Hensen I. (2010) Do *Polylepis australis* trees tolerate herbivory? Seasonal patterns of shoot growth and its consumption by livestock. *Plant Ecol.* **207**, 307–19.
- Gross N., Suding K. N. & Lavorel S. (2007) Leaf dry matter content and lateral spread predict response to land use change for six subalpine grassland species. *J. Veg. Sci.* **18**, 289–300.
- Harrison M. T., Kelman W. M., Moore A. D. & Evans J. R. (2010) Grazing winter wheat relieves plant water stress and transiently enhances photosynthesis. *Funct. Plant Biol.* **37**, 726–36.
- Hendry G. A. F. & Grime J. P. (1993) *Methods in comparative plant ecology, a laboratory manual*. Chapman & Hall, London, UK.
- Huntly N. (1991) Herbivores and the dynamics of communities and ecosystems. *Annu. Rev. Ecol. Syst.* **22**, 477–503.
- Jacobsen A. L., Pratt R. B., Davis S. D. & Ewers F. W. (2008) Comparative community physiology: nonconvergence in water relations among three semi-arid shrub communities. *New Phytol.* **180**, 100–13.
- Kalapos T. (1994) Leaf water potential–leaf water deficit relationship for ten species of a semiarid grassland community. *Plant Soil* **160**, 105–12.
- Kohler F., Gillet F., Reust S., *et al.* (2006) Spatial and seasonal patterns of cattle habitat use in a mountain wooded pasture. *Landsc. Ecol.* **21**, 281–95.
- Kotaniemi P. M. & Bergelson J. (2000) Effects of simulated grazing on different genotypes of *Bouteloua gracilis*: how important is morphology? *Oecologia* **123**, 66–74.
- Lavorel S. & Garnier E. (2002) Predicting changes in community composition and ecosystem functioning from plant traits. *Funct. Ecol.* **16**, 545–56.
- Lavorel S., de Bello F., Grigulis K., *et al.* (2011) Response of herbaceous vegetation functional diversity to land use change across five sites in Europe and Israel. *Isr. J. Ecol. Evol.* **57**, 53–72.
- Leishman M. R., Haslehurst T., Ares A. & Baruch Z. (2007) Leaf trait relationships of native and invasive plants: community- and global-scale comparisons. *New Phytol.* **176**, 635–43.
- Loreti J., Oosterheld M. & Sala O. (2001) Lack of intraspecific variation in resistance to defoliation in a grass that evolved under light grazing pressure. *Plant Ecol.* **157**, 195–202.
- McIntyre S. & Lavorel S. (2001) Livestock grazing in sub-tropical pastures: steps in the analysis of attribute response and plant functional types. *J. Ecol.* **89**, 209–26.
- McNaughton S. J. (1979) Grazing as an optimization process: grass–ungulate relationships in the Serengeti. *Am. Nat.* **113**, 691–703.
- McNaughton S. J. (1984) Grazing lawns: animals in herds, plant form, and coevolution. *Am. Nat.* **124**, 863–86.
- Nowak R. S. & Caldwell M. M. (1984) A test of compensatory photosynthesis in the field: Implications for herbivory tolerance. *Oecologia* **61**, 311–8.
- Oosterheld M. & McNaughton S. J. (1988) Intraspecific variation in the response of *Themeda triandra* to defoliation: the effect of time of recovery and growth rates on compensatory growth. *Oecologia* **77**, 181–6.
- Pérez-Harguindeguy N., Díaz S., Garnier E., *et al.* (2013) New handbook for standardised measurement of plant functional traits worldwide. *Aust. J. Bot.* **61**, 167–234.
- Pittermann J., Lance J., Poster L., Baer A. & Fox L. R. (2014) Heavy browsing affects the hydraulic capacity of *Ceanothus rigidus* (Rhamnaceae). *Oecologia* **175**, 801–10.
- R Development Core Team (2012) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. Available from URL: <http://www.R-project.org>
- Reich P. B. (2014) The world-wide “fast–slow” plant economics spectrum: a traits manifesto. *J. Ecol.* **102**, 275–301.
- Reich P. B., Wright I. J., Cavender-Bares J., *et al.* (2003) The evolution of plant functional variation: traits, spectra, and strategies. *Int. J. Plant. Sci.* **164**, 143–64.
- Reisch C. & Poschold P. (2003) Intraspecific variation, land use and habitat quality – a phenologic and morphometric analysis of *Sesleria albicans* (Poaceae). *Flora – Morphol. Distrib. Funct. Ecol. Plants* **198**, 321–8.
- Rotundo J. L. & Aguiar M. R. (2008) Herbivory resistance traits in populations of *Poa ligularis* subjected to historically different sheep grazing pressure in Patagonia. *Plant. Ecol.* **194**, 121–33.
- Rusch G. M., Skarpe C. & Halley D. J. (2009) Plant traits link hypothesis about resource-use and response to herbivory. *Basic Appl. Ecol.* **10**, 466–74.
- Schlichting C. D. (1986) The evolution of phenotypic plasticity in plants. *Annu. Rev. Ecol. Syst.* **17**, 667–93.
- Scholander P. F., Bradstreet E. D., Hemmingen E. A. & Hammel H. T. (1965) Sap pressure in vascular plants negative hydrostatic pressure can be measured in plants. *Science* **148**, 339–46.
- Shipley B., Vile D. & Garnier E. (2006) From plant traits to plant communities: a statistical mechanistic approach to biodiversity. *Science* **314**, 812–4.
- Siefert A., Violle C., Chalmandrier L., *et al.* (2015) A global meta-analysis of the relative extent of intraspecific trait variation in plant communities. *Ecol. Lett.* **18**, 1406–19.
- Stobbs T. H. (1973) The effect of plant structure on the intake of tropical pastures. I. Variation in the bite size of grazing cattle. *Aust. J. Agric. Res.* **24**, 809–19.
- Svejcar T. & Christiansen S. (1987) The influence of grazing pressure on rooting dynamics of *Caucasian bluestem*. *J. Range Manag.* **40**, 224–7.
- Tomás M. A., Carrera A. D. & Poverene M. (2000) Is there any genetic differentiation among populations of *Piptochaetium napostaense* (Speg.) Hack (Poaceae) with different grazing histories? *Plant Ecol* **147**, 227–35.
- Vaieretti M. V., Díaz S., Vile D. & Garnier E. (2007) Two measurement methods of leaf dry matter content produce similar results in a broad range of species. *Ann. Bot.* **99**, 955–8.
- Valladares F., Gianoli E. & Gómez J. M. (2007) Ecological limits to plant phenotypic plasticity. *New Phytol.* **176**, 749–63.
- Vesk P. A. & Westoby M. (2001) Predicting plant species’ responses to grazing. *J. Appl. Ecol.* **38**, 897–909.
- Violle C., Enquist B. J., McGill B. J., *et al.* (2012) The return of the variance: intraspecific variability in community ecology. *Trends Ecol. Evol.* **27**, 244–52.
- Von Müller A. R., Cingolani A. M., Vaieretti M. V. & Renison D. (2012) Estimación de carga bovina localizada a partir de frecuencia de deposiciones en un pastizal de montaña. *Ecol. Austral* **22**, 178–87.
- Wang L., Liu H., Ketzner B., Horn R. & Bernhofer C. (2012) Effect of grazing intensity on evapotranspiration in the semi-arid grasslands of Inner Mongolia. *China. J. Arid Environ.* **83**, 15–24.

- Westoby M. (1980) Relations between genet and tiller population dynamics: survival of *Phalaris tuberosa* tillers after clipping. *J. Ecol.* **68**, 863–9.
- Wright I. J., Falster D. S., Pickup M. & Westoby M. (2006) Cross-species patterns in the coordination between leaf and stem traits, and their implications for plant hydraulics. *Physiol. Plant.* **127**, 445–56.
- Wright I. J., Reich P. B., Westoby M., *et al.* (2004) The worldwide leaf economics spectrum. *Nature* **428**, 821–7.
- Zeballos S. R., Giorgis M. A., Cingolani A. M., Cabido M., Whitworth-Hulse J. I. & Gurvich D. E. (2014) Do alien and native tree species from Central Argentina differ in their water transport strategy? *Austral Ecol.* **39**, 984–91.