

Phenotypic plasticity as an index of drought tolerance in three Patagonian steppe grasses

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• **Background and Aims** Despite general agreement regarding the adaptive importance of plasticity, evidence for the role of environmental resource availability in plants is scarce. In arid and semi-arid environments, the persistence and dominance of perennial species depends on their capacity to tolerate drought: tolerance could be given on one extreme by fixed traits and, on the other, by plastic traits. To understand drought tolerance of species it is necessary to know the plasticity of their water economy-related traits, i.e. the position in the fixed–plastic continuum.

• **Methods** Three conspicuous co-existing perennial grasses from a Patagonian steppe were grown under controlled conditions with four levels of steady-state water availability. Evaluated traits were divided into two groups. The first was associated with potential plant performance and correlated with fitness, and included above-ground biomass, total biomass, tillering and tiller density at harvest. The second group consisted of traits associated with mechanisms of plant adjustment to environmental changes and included root biomass, shoot/root ratio, tiller biomass, length of total elongated leaf, length of yellow tissue divided by time and final length divided by the time taken to reach final length.

• **Key Results and Conclusions** The most plastic species along this drought gradient was the most sensitive to drought, whereas the least plastic and slowest growing was the most tolerant. This negative relationship between tolerance and plasticity was true for fitness-related traits but was trait-dependent for underlying traits. Remarkably, the most tolerant species had the highest positive plasticity (i.e. opposite to the default response to stress) in an underlying trait, directly explaining its drought resistance: it increased absolute root biomass. The niche differentiation axis that allows the coexistence of species in this group of perennial dryland grasses, all limited by soil surface moisture, would be a functional one of fixed versus plastic responses.

Key words: Dryland, perennial grasses, mechanistic traits, performance traits, trade-off, *Bromus pictus*, *Poa ligularis*, *Pappostipa speciosa*.

INTRODUCTION

Natural environments are variable in space and time; the change in an organism's phenotype triggered by such variations is known as phenotypic plasticity (Bradshaw, 1965). The plasticity of a trait depends on the actual trait, the environmental gradient and the genotype (Trewavas, 2003). Plasticity for a given trait may have important adaptive effects, minimizing deleterious effects of the environment and maximizing survival, growth and reproduction, but it may also be maladaptive or neutral (De Witt *et al.*, 1998; Alpert and Simms, 2002; Van Kleunen and Fischer, 2005; Valladares and Niinemets, 2008).

Differences in plasticity for the same trait across environments could be determined by the different selective pressures they impose. In stable environments, plasticity would not be indispensable as a fixed phenotype would be well adjusted to the most likely conditions (Alpert and Simms, 2002; Callaway *et al.*, 2003; Gianoli and González-Teuber, 2005; Valladares *et al.*, 2007). As long as mechanisms and structures that trigger and allow a plastic response have some kind of

cost, paying this cost would be unnecessary most of the time (Steinger *et al.*, 2003). Such a cost would be maladaptive in poor environments, whereas in rich environments it is less likely to affect fitness (Alpert and Simms, 2002). At least for plants, the experimental evidence for the connection between environmental resource availability and plasticity is scarce (Alpert and Simms, 2002). This seems to be especially true for water shortages.

To understand species' capacity to persist in stressful environments, it is important to understand their ability to tolerate resource limitation. A species is defined as more tolerant when stress has a lower effect on its performance (e.g. growth and therefore biomass), to the point at which it may seem that it is not perceiving the stress. Such apparent lack of effect on fitness, however, could be explained by changes in other traits, less visible and more distant to fitness (underlying traits *sensu* Alpert and Simms, 2002, and functional traits *sensu* Violle *et al.*, 2007). In other words, stress always has an effect at some level: sometimes at the 'macroscopic' level, affecting fitness, and sometimes only at a more detailed level, modifying underlying traits that allow fitness stability.

This distinction appears to answer Weiner's (2004) plea for a refinement of the plasticity concept and seems worthy of investigation.

According to the trade-off model, there is a compromise between the ability to grow under benign environmental conditions and the ability to tolerate resource limitation (Lambers *et al.*, 2008). Inherently fast-growing species (*sensu* Grime, 1979; Lambers and Poorter, 2004) are more strongly affected by stress than typical tolerant species. The trade-off is evidenced through their phenotypic responses to an environmental gradient (norm of reaction, see below), as a differential response between fast-growing species and tolerant species; statistical evidence for this is provided by the significance of the interaction between environment and species. The alternative no-trade-off model is that both species respond similarly to stress, with no significant interaction.

In arid and semi-arid environments, the persistence and dominance of perennial species depend on their capacity to tolerate drought. Tolerance could be given on one extreme by fixed traits and, on the other, by plastic traits. This can even occur for the same trait: allocation to the root system, for example, would be expected to be inherently high (fixed) in drought-adapted species, and also to be increased (plastic) in response to stress. This is not the same as saying that plasticity is always adaptive; for example, a decrease in total plant biomass caused by stress is not adaptive. To understand drought tolerance of species it is necessary to know the plasticity of their water economy-related traits (i.e. position in the fixed–plastic continuum).

The western Patagonian steppe has been intensively studied since about 1950 as a model of a semi-arid system (Soriano, 1956; León *et al.*, 1998). Perennial grasses and shrubs are the dominant functional plant types in the area. These groups have differences in water use associated with differences in structural and functional traits (Soriano and Sala, 1983; Sala *et al.*, 1989). Perennial C₃ grasses constitute the most homogeneous, physiologically tolerant and abundant functional type in this habitat (representing two-thirds of net primary productivity; Fernández *et al.*, 1991; Golluscio *et al.*, 2005). These species show conspicuous homogeneity of root distribution (all species have higher root density in the first 30 cm of the soil profile; Soriano and Sala, 1983; Soriano *et al.*, 1987; Golluscio *et al.*, 2005) and phenology (mainly linked to precipitation regime and water availability; Golluscio *et al.*, 2005). Although they differ in distribution and abundance (Soriano, 1956; Golluscio *et al.*, 1982; León *et al.*, 1998), these species use water from the same part of the soil profile at the same time, suggesting a strong niche overlap; how then do we explain their coexistence in an environment mainly limited by a single resource (Gause, 1934)? This might be answered by divergence in other traits, such as root anatomy (Leva *et al.*, 2009) and perhaps function, litter chemistry (Vivanco and Austin, 2006), palatability (Soriano, 1956; Golluscio *et al.*, 1998), seed dispersion, safe-site requirement for recruitment (Fernández *et al.*, 2002; Semmartin *et al.*, 2004; Cipriotti and Aguiar, 2005) and capacity to recover after drought (Yahdjian and Sala, 2006). Most of these characteristics are associated with a higher capacity to tolerate drought, i.e. are xeromorphic traits (see Materials and Methods); thus, they suggest that there could

be interspecific differences in physiological tolerance to drought. However, there are no published experimental data on this. Here, we provide such evidence using controlled-environment methods devised for comparisons of inter-specific tolerance, controlling for intrinsic and drought-induced differences in plant biomass.

The main goal was to evaluate the degree of drought tolerance differentiation between the most conspicuous dryland bunchgrass species as an explanation for their coexistence. We propose that their phenotypic plasticity in traits associated with water use can be used as an index of drought tolerance. We constructed 'norm of reaction'-type graphs that represent the phenotypic changes (y-axis) due to changes in the environment (x-axis), with the slope representing phenotypic plasticity. As a given genotype may have high plasticity for one trait and low plasticity for another, we evaluated traits at two levels of organization. At the macroscopic level, the one closer to fitness, we measured 'performance traits': plant-level growth and resulting biomass accumulation. At the more detailed level, the underlying one, we measured 'mechanistic traits': biomass partitioning and organ-level growth (analogous to 'performance traits' and 'functional traits', respectively, *sensu* Violle *et al.*, 2007).

The main hypothesis was that there is a trade-off between tolerance to drought and growth under less limiting conditions, but that this traditional model only applies to performance traits, i.e. those at the macroscopic level. The secondary hypothesis was that the opposite occurs at the more detailed level: here, the more tolerant species are more plastic in mechanistic traits, i.e. those that allow them more constancy in fitness.

MATERIALS AND METHODS

Plant material

To study the plastic response of perennial grass species to changes in water availability, an experiment was developed under controlled conditions. These grass species coexist in the Occidental Patagonian District of the Patagonian steppe. This semi-arid community has been called 'coironal' because of the dominance of perennial bunchgrasses, locally called 'coirones' (*Pappostipa* species, chiefly *P. speciosa*; Soriano, 1956; Golluscio *et al.*, 1982). The dominant shrubs in the community are *Mulinum spinosum* (Cav.) Pers., *Adesmia volkmanni* Phil. and *Senecio filaginoides* DC. Among the most conspicuous grasses, representing 80% of the biomass in the guild, are *Pappostipa speciosa* (Trin. and Rupr.) Romansch., *Poa ligularis* Nees ap. Steud. and *Bromus pictus* Hook (Golluscio *et al.*, 1982; León *et al.*, 1998; Golluscio and Oesterheld, 2007); these three species were evaluated here. They multiply mostly vegetatively by tillering; seedlings are extremely rare, except for *Poa ligularis* (Fernández *et al.*, 2002). Based on their xeromorphic characteristics (Table 1), it is possible that these species have different abilities to tolerate drought.

The seeds used in the experiment were collected during summer 2008 at the INTA Rio Mayo Experimental Station (45°41'S, 70°16'W), in a typical coironal community. Mean annual precipitation at the site is 136 mm and ranged between 47 and 230 mm (37 years of data; Fernández *et al.*,

TABLE 1. Xeromorphism comparison between three Patagonian grasses based on the literature: nitrogen content of litter [N (%)], lignin contents of litter [lignin (%)], lignin–nitrogen litter ratio (lignin/N), decomposition rate (K), carbon–nitrogen leaf ratio (C/N leaf), cover and frequency percentage of occurrence of species and palatability

Characteristic	Patagonian grass species			Source
	<i>B. pictus</i>	<i>Po. ligularis</i>	<i>Pa. speciosa</i>	
N (%)	0.61	0.41	0.41	Semmartin <i>et al.</i> (2004)
Lignin (%)	8.85	8.12	11.47	Semmartin <i>et al.</i> (2004)
Lignin/N	14.51	19.80	27.98	Semmartin <i>et al.</i> (2004)
K (year ⁻¹)	0.6	0.52	0.51	Semmartin <i>et al.</i> (2004)
C/N leaf	33.9	61.9	63.5	Adler <i>et al.</i> (2004)
Cover (%)	1.28	3.56	12.69	Golluscio <i>et al.</i> (1982)
Frequency (%)	75	98	98	Golluscio <i>et al.</i> (2005)
Palatability	higher	intermediate	smaller	Soriano (1956)
Xeromorphism	low	intermediate	high	Golluscio <i>et al.</i> (1998) inferred from above

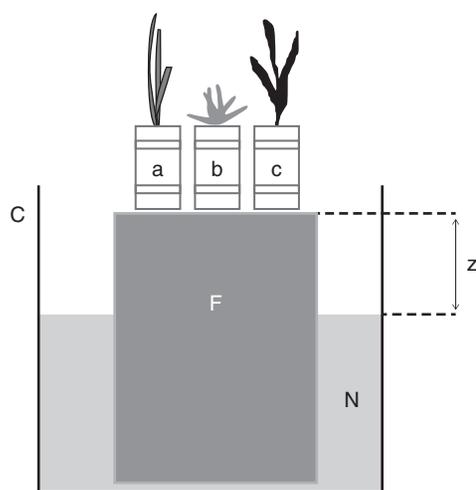


FIG. 1. Drought device, based on Snow and Tingey (1985). Abbreviations: C, plastic container; F, hygroscopic commercial foam; N, nutrient solution; a–c, sand-filled pots with a permeable base (polyester fabric); z, water-table depth (level of water stress). Redrawn from Fernández and Reynolds (2000).

1991), falling mainly during autumn and winter (Jobbágy and Sala, 2000). The seeds were placed in germination chambers at 15 °C in the dark; after emergence, they were transplanted to individual plastic pots filled with sand. Plants were irrigated daily with nutrient solution to reach a size of two tillers and then assigned to one of four drought treatments.

Drought treatment

Given the potential effects of size/biomass on water stress in potted plants (e.g. Ray and Sinclair, 1998; Passioura, 2006; Fernández, 2010) we used a method specifically devised to avoid the negative effect of plant uptake on soil-water availability (i.e. to measure plants responses independently of plant effects, *sensu* Goldberg, 1990). This was a sub-irrigation technique which allows constant and uniform water potential in pots (Fig. 1). It was first proposed by Haan and Barfield (1971) and then developed by Snow and Tingey (1985) and

used in several studies (e.g. Fernández and Reynolds, 2000; Couso *et al.*, 2010). Its main advantage is ensuring an unlimited access to water at fixed availability and independently of plant size (Wookey *et al.*, 1991; Saulescu *et al.*, 1995). The device comprises a column of hygroscopic foam (porous commercial Styrofoam; Smithers-Oasis, OH, USA) along which the water rises through capillary action to the base of each pot (Fig. 1). Individual plants were grown in pots sitting on these columns, which in turn were placed inside large plastic containers. The pot bases were permeable to the upward water flux. The water depth determined the distance from the water surface to the base of the pots (a sort of ‘water table depth’), and thus the level of water availability to plants.

Experimental design and measurements

Plants of the three species studied were subjected to four levels of drought during 60 d of growth in a greenhouse, split-plot experiment. The main plots corresponded to water supply treatment (four levels). The sub-plots corresponded to perennial grass species (three levels: *Bromus pictus*, *Poa ligularis* and *Pappostipa speciosa*). The main treatment was imposed on 16 plastic containers (4 water levels × 4 replicates arranged in blocks). Eighteen pots were placed in each container on top of the Styrofoam columns as explained above (18 = 6 pots or subsamples × 3 species, representing the species variability). The total number of pots in the experiment was 288 (18 pots × 4 drought levels × 4 blocks). The four levels of water availability (water table depth) were: 6 cm (control), 13 and 20 cm (mild-drought conditions), and 27 cm (drought condition). Each of the four plastic containers from each block was randomly assigned to one level of water supply. A diluted standard nutrient solution was used to ensure that water was the only limiting factor. The pots had been fitted at their base with a permeable cloth and filled with sand.

In the glasshouse, located in our Buenos Aires campus, maximum photosynthetically active radiation at 1200 h was 825 $\mu\text{mol m}^{-2} \text{s}^{-1}$, and daily temperatures ranged between 25 and 10 °C. Three tillers of each plant and three leaves of each tiller were identified and marked during the experiment (the total number of marked tillers was 1152 and leaves 3456).

The number of live tillers, length of marked leaves (green and yellow) and number of live leaves were measured once a week until harvest. At harvest (day 60), we measured above-ground biomass (g), root biomass (g) and tiller density (tiller per pot). These data were collected for each pot and seven variables were then calculated: leaf elongation rate (LER, mm leaf d⁻¹), leaf senescence rate (LSR, mm new yellow tissue d⁻¹), final lamina length (FLL, mm), tillering (number of new tillers d⁻¹), tiller biomass (g per tiller), total biomass (g) and shoot/root biomass ratio (see Fig. 2).

Studied traits

Evaluated traits were included in two different groups (Fig. 2). The first group comprised traits associated with potential plant performance and correlated with fitness, and were called ‘performance traits’ (*sensu* Violle *et al.*, 2007): above-ground biomass, total biomass (above-ground and root biomass), tillering (number of new tillers per day) and tiller density at harvest. The second group included traits associated with mechanisms of plant adjustment to environmental changes – water changes in this case (functional traits *sensu* Violle *et al.*, 2007). The latter were called ‘mechanistic traits’: root biomass, shoot/root biomass ratio, tiller biomass (total biomass divided by number of tillers), FLL (length of total elongated leaf), LSR (length of yellow tissue divided

by time) and LER (final length divided by the time it takes to reach the final length; Bahmani *et al.*, 2000; Lemaire and Agnusdei, 2000; Bultynck *et al.*, 2004).

Statistical and graphical analyses

Results were analysed using the ‘InfoStat’ statistical package (Universidad Nacional de Córdoba, Argentina). Analysis of variance (ANOVA) and Tukey mean comparison tests were performed with a significance level of 5%. ANOVA assumptions were checked, and total biomass, above-ground biomass and tiller biomass were square-root transformed. The interaction between block and treatment was always non-significant. Therefore, normality, homogeneity of variance and block–treatment additivity assumptions were fulfilled. Results were graphically presented as ‘norms of reaction’ (see above). Species with similar slopes in a norm of reaction have similar plasticity for the trait considered, indicating that there is no trade-off between performance and tolerance for this trait (Sambatti and Caylor, 2007). The results are shown as graphic ‘reaction norms’ for the sake of presentation, but the corresponding statistical analyses are summarized in Table 2.

To summarize our findings, three indices of plasticity (IP, *sensu* Valladares *et al.*, 2000) were calculated for each species. The first index included only performance traits, the second index included mechanistic traits and the third index included all the studied traits.

$$IP = (M_{\max} - M_{\min})/M_{\max}$$

where M_{\max} is the treatment average with highest values (control) and M_{\min} the treatment average with lowest values (drought condition).

RESULTS

The three traits evaluated at the leaf level (LER, LSF, FLL) showed a significant interaction between drought and species (Table 2, Fig. 3): values decreased with increased drought for all three species, but in different proportion. *Bromus pictus* had the highest trait values under non-limiting conditions (Fig. 3); however, with greater drought stress, LER, LSF and FLL decreased to values resembling those of the other species. Average values for these traits for *B. pictus* were also higher than for the other two species. *B. pictus* was the species most affected by drought (higher slope in its norm of reaction; Fig. 3), followed by *Po. ligularis* and then *Pa. speciosa*.

Tillering, and thus tiller density, also showed a significant interaction between drought and species; *Pa. speciosa* was clearly less affected by drought than the other species (Table 2, Fig. 4B). On the other hand, tiller biomass differed between species but responded to drought in a similarly negative way in each of them (interaction not significant; Table 2, Fig. 4A). As for the leaf-level traits, *B. pictus* again showed the highest average value for tiller biomass (five times higher) and *Pa. speciosa* showed the lowest average. Tiller biomass responded to drought in the same way in all three species: they presented a similar slope, i.e. a similar plasticity.

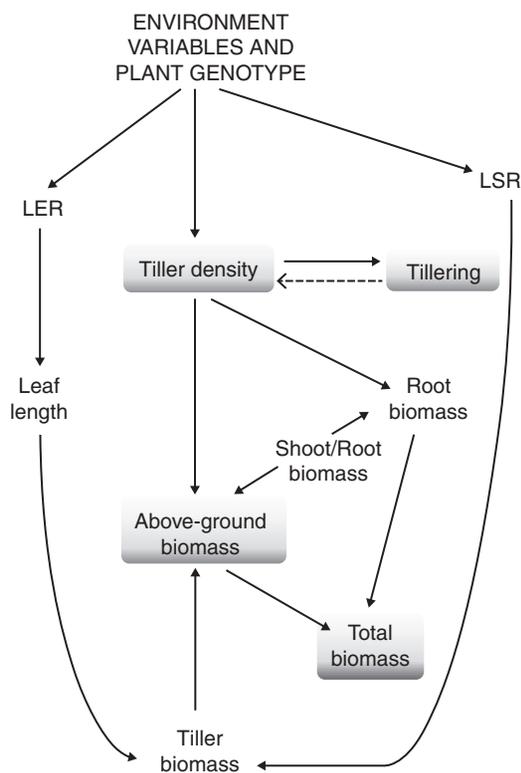


FIG. 2. Evaluated traits on leaf, tiller and whole-plant level and their relationship (modified from Chapman and Lemaire, 1993 and Hirata and Pakiding, 2002). The figure includes ‘performance traits’, traits directly associated with fitness (in frames) and ‘mechanistic traits’, traits directly associated with mechanical response when the plants are subjected to drought conditions (without frames). LER, leaf elongation rate; LSR, leaf senescence rate.

TABLE 2. ANOVA results

Level	Variable	Drought effect		Species effect		Drought × species	
		F	P	F	P	F	P
Leaf	Leaf elongation rate	16.61	<0.01**	67.68	<0.01**	3.79	<0.01**
	Leaf senescence rate	5.34	0.02*	20.55	<0.01**	6.95	<0.01**
	Final leaf length	14.57	<0.01**	36.76	<0.01**	12.68	<0.01**
	Tiller biomass	3.25	0.07	232.28	<0.01**	1.51	0.18
Tiller	Tillering	29.5	<0.01**	26.3	<0.01**	2.72	0.016*
	Tiller density	32.07	<0.01**	24.38	<0.01**	2.74	0.015*
	Above-ground biomass	12.39	<0.01**	78.89	<0.01**	5.61	<0.01**
Whole plant	Root biomass	0.51	0.69	40.45	<0.01**	2.02	0.07
	Total biomass	6.2	0.014**	63.25	<0.01**	5.94	<0.01**
	Shoot/root biomass	15.04	<0.01**	26.17	<0.01**	0.82	0.6

Responses of three Patagonian grass species to four levels of drought and their interaction with it. Significance levels: * $P < 0.05$; ** $P < 0.001$.

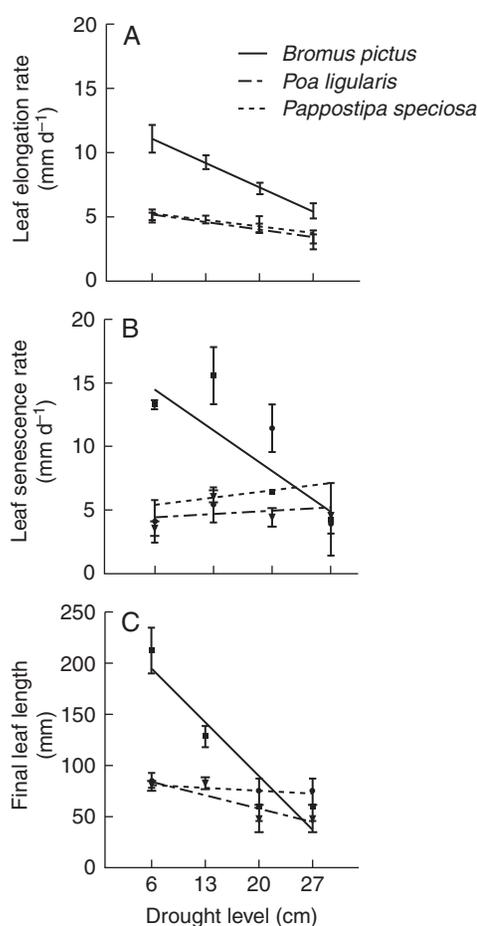


FIG. 3. Norms of reaction at the leaf level. Graphs represent the response to drought level (means \pm s.e.) (6 cm: control, 13–20 cm: mild drought conditions, 27 cm: drought condition) for three Patagonian grasses: *Bromus pictus*, *Poa ligularis* and *Pappostipa speciosa*. (A) Leaf elongation rate, (B) leaf senescence rate and (C) final leaf length.

Tiller density and tillering showed a different ranking than for the other studied traits: averaged across treatments, the highest values corresponded to *Po. ligularis* and the lowest to *B. pictus*. *Pa. speciosa* was similar to *B. pictus* in the control treatment but similar to *Po. ligularis* under drought

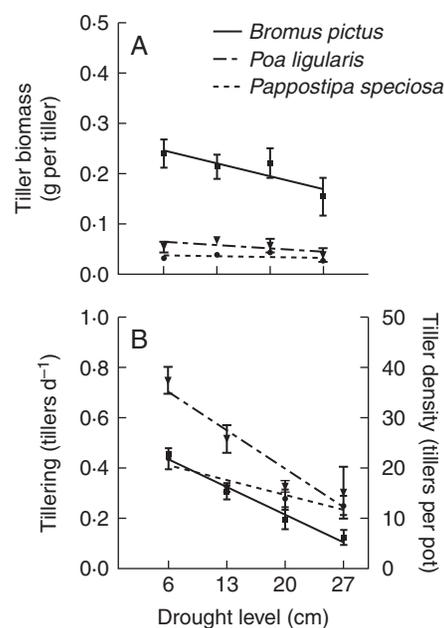


FIG. 4. Norms of reaction at the tiller level. Graphs represent the response to drought level (means \pm s.e.) (6 cm: control, 13–20 cm: mild drought conditions, 27 cm: drought condition) for three Patagonian grasses: *Bromus pictus*, *Poa ligularis* and *Pappostipa speciosa*. (A) Tiller biomass and (B) tillering and tiller density.

conditions. In addition, *Pa. speciosa* had the lowest slope in its response, i.e. the lowest plasticity in tiller density and tiller production rate.

At the whole-plant level, both total and above-ground biomass had a significant interaction between drought and species (Table 2, Fig. 5A, C). Under well-watered conditions there were large differences between species, with *B. pictus* having the highest values, but these differences faded as drought intensified. *Po. ligularis* showed intermediate values for above-ground biomass while in total biomass showed similar values to those of *Pa. speciosa*. Shoot/root ratio differed between species and drought treatments but did not have a significant interaction between them (Fig. 5D). Root biomass presented a marginally significant drought \times species interaction ($P = 0.07$): there were more differences between

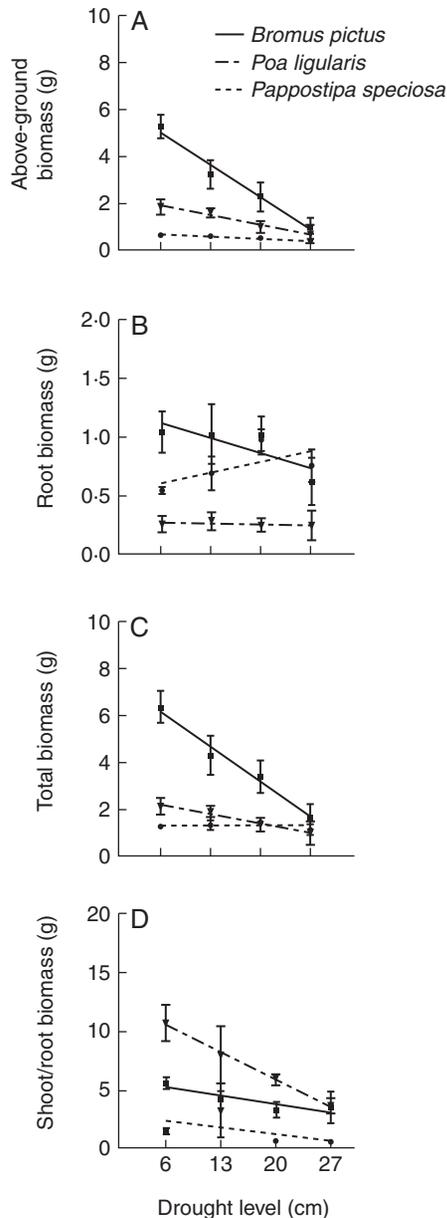


FIG. 5. Norms of reaction at the whole-plant level. Graphs represent the response to drought level (means \pm s.e.) (6 cm: control, 13–20 cm: mild drought conditions, 27 cm: drought condition) for three Patagonian grasses: *Bromus pictus*, *Poa ligularis* and *Pappostipa speciosa*. (A) Above-ground biomass, (B) root biomass, (C) total biomass and (D) shoot/root biomass.

species than between treatments (Table 2, Fig. 5B). Root biomass of *Po. ligularis* showed overall low values and *Pa. speciosa* always showed the highest values. For the same trait, a decreasing tendency was found in *B. pictus* when drought increased. Conversely, *Pa. speciosa* increased root biomass when drought increased.

As found for most previous traits, *B. pictus* presented the highest average values in total biomass, above-ground biomass and root biomass. *Pa. speciosa* showed, in all whole-plant traits except root biomass, the lowest values across treatments; in most cases, its slope against drought did not differ from zero, showing the lowest plasticity for these traits.

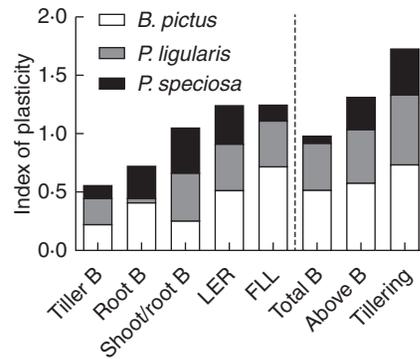


FIG. 6. Index of plasticity [$IP = (M_{\max} - M_{\min}) / M_{\max}$; see text] for each trait for each Patagonian grass species: *Bromus pictus*, *Poa ligularis* and *Pappostipa speciosa*. Left side (mechanistic traits): tiller biomass, root biomass, shoot/root biomass, leaf elongation rate (LER) and final leaf length (FLL). Right side (performance traits): total biomass, above-ground biomass, tillering (rate and density). B = biomass.

Shoot/root biomass, like tiller biomass, was the only trait with non-significant or marginally significant interactions between drought and species, and only the first one had a drought main effect. Despite large responses in total biomass (significant species main effect, $P < 0.01$), all three species showed fairly constant tiller biomass along the water supply gradient. High plasticity in tiller density seems to be the way to preserve tiller biomass along the gradient.

The IP for each single trait (Fig. 6) revealed tiller biomass and root biomass as the least plastic traits, and tiller density as the most plastic trait. Thus, the response of the three species under stress conditions is readily observed (at the more visible level: performance trait) through adjustment of tiller density. Regarding mechanistic traits, leaf length showed high plasticity, with values similar to those of above-ground biomass (a performance trait). At the same time, plasticity of root biomass (the most fixed trait) was highest for *B. pictus* and *Pa. speciosa*, species at opposite ends of the gradient. In the case of *B. pictus* increased drought stress resulted in a decrease of root biomass, in contrast to the result for *Pa. speciosa*. The latter situation is only observed for *Pa. speciosa*, and can be called ‘positive plasticity’.

Unlike those for mechanistic traits that presented only tendencies in their responses, IPs for performance traits showed differences between species (Fig. 7A, B). For both types of traits, the IP ranking was the same: *B. pictus* with highest values followed by *Po. ligularis* and *Pa. speciosa*. Accordingly, both IPs combined into one index showed the same results (Fig. 7C). Based on this combined index, *Pa. speciosa* was the least plastic species and *B. pictus* was the highest for both types of traits. *B. pictus*, the most plastic species, shows greater plasticity in performance traits than in mechanistic traits. In turn, *Pa. speciosa* showed similar values in performance and mechanistic traits, and in the latter values included one case of positive plasticity (as defined above: root biomass).

A performance measure unequivocally related to fitness is the proportion of dead plants for each drought level. The overall most plastic species, *B. pictus*, was also the most sensitive to drought: the number of dead plants increased with greater drought stress. At the other extreme, the least plastic

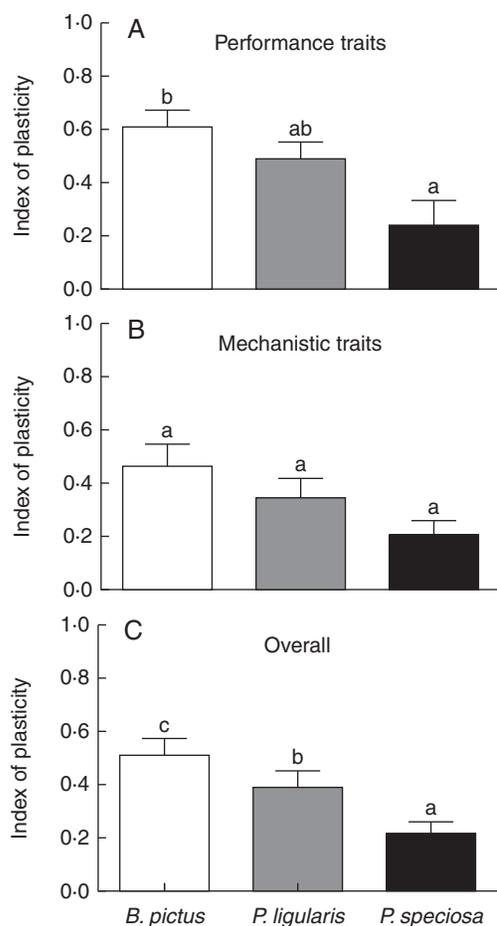


FIG. 7. Index of plasticity [$IP = (M_{max} - M_{min})/M_{max}$; see text] for each trait for each Patagonian grass species: *Bromus pictus*, *Poa ligularis* and *Pappostipa speciosa*. (A) Plasticity for performance traits: total biomass, above-ground biomass and tillering ($P = 0.033$). (B) Plasticity for mechanistic traits: tiller biomass, root biomass, shoot/root biomass, leaf elongation rate and final leaf length ($P = 0.075$). (C) Overall plasticity calculated with all traits included in both categories ($P = 0.003$). Different letters indicate significant differences between means ($P < 0.05$).

species, *Pa. speciosa*, was the least sensitive to drought: death rates were similar across the entire drought gradient. The proportion of *Po. ligularis* dead plants was intermediate (Fig. 8).

DISCUSSION

As expected from the trade-off model, these three Patagonian species showed different responses to drought for most performance traits (as evidenced by statistically significant species-by-stress interactions). This supports our first hypothesis and confirms the suggestion of their different degree of stress tolerance. Despite the information available for various species (see Introduction and Table 1), and considering the precautions taken to avoid a positive correlation between plant biomass and water stress (see Drought Treatment), this seems to be the first study to provide conclusive results. Moreover, previous inter- and intra-specific studies associated with water on C_4 perennial grass species have shown a lack of trade-offs (Fernández and Reynolds, 2000; Couso et al., 2010).

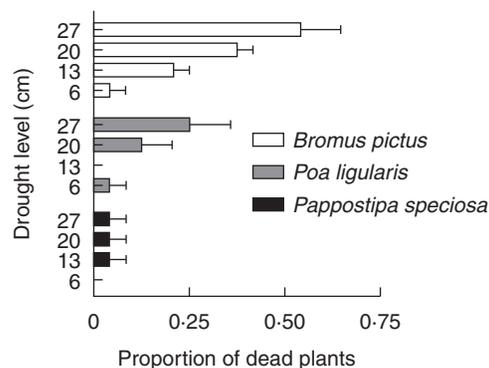


FIG. 8. Proportion of dead plants for each Patagonian grass species (*Bromus pictus*, *Poa ligularis* and *Pappostipa speciosa*) at four different drought levels (here increasing upwards within each species) in the complete experimental period ($P = 0.008$).

B. pictus was the most sensitive to drought out of the three species. Under close to optimal conditions it showed the highest total and above-ground biomass, values that were reduced by more than 50% under drought stress. A comparable relative reduction was observed for tiller number, with a slope similar to that for *Po. ligularis* and steeper than for *Pa. speciosa*. In addition, *B. pictus* had the highest potential for improving performance traits, and then in relative terms it would fit into a water-spending, or Grime's (1979), competitive strategy. This is consistent with the high senescence rate of *B. pictus*, revealing the rapid tissue turnover associated with a high maximal relative growth rate, RGR_{max} (Reich, 1998). Interestingly, senescence was plastic in a counter-intuitive direction, leading to higher leaf longevity under drought. This could be interpreted as an evolutionary response to stabilize leaf area, and thus carbon gain, in the face of low biomass production, as has been observed in *Cryptantha flava*, another herbaceous perennial of arid zones (Casper et al., 2001), and also in trees (Leuzinger et al., 2005).

At the other extreme, *Pa. speciosa* was the most tolerant species, with almost constant total biomass across the water availability range (slope undistinguishable from zero; $P = 0.93$). Above-ground biomass and tiller number did decrease, but only gently. *Pa. speciosa* thus clearly fits a water-saving, or Grime's (1979) stress-tolerant, strategy. Therefore, the slow-growing species showed the lowest effect of drought on above-ground and total biomass in comparison with the two fast-growing species (similar to *Miscanthus* grass genotypes; Clifton-Brown and Lewandowski, 2000). For *Po. ligularis*, biomass (both for average values and in response to stress) was intermediate between that of the other two species. However, its tillering rate and density experienced the highest reduction under stress compared with the other two species. This was consistent with *Po. ligularis* exhibiting the largest absolute values for these variables, and fit the general trade-off pattern: the highest a trait value under close to optimum conditions, the stronger the detrimental effect of drought. The tolerance ranking for the three C_3 perennial grasses of this Patagonian steppe was, thus $Pa. speciosa > Po. ligularis > B. pictus$. The overall plasticity ranking, as shown by Fig. 7C, was the opposite, with the most tolerant

species being the least plastic in performance traits, as expected from our first hypothesis.

The question, then, regarding our second hypothesis is whether this low plasticity in performance traits observed for tolerant species is mediated by a higher plasticity in the least visible traits associated with response mechanisms. The answer, indicated by the coincident rankings for performance traits, mechanistic traits and therefore overall plasticity, is a clear 'no'. In fact, plants responding more to changes in their environment at the visible scales show parallel and less apparent comparable changes occurring at the same time and probably explaining such a response. If a lack of plasticity at all levels characterizes tolerant species, this begs the question of whether possessing fairly fixed traits is the most adaptive way to thrive in dry environments. Answering this is not straightforward, or at least requires us to specify the type of trait, as the high tolerance (and generally low plasticity) of *Pa. speciosa* seems to be explained by the high plasticity of the two mechanistic traits describing allocation: root biomass and shoot/root biomass.

Returning to the general question of coexistence of plant species, particularly of species belonging to the same functional group and apparently competing for one single resource, we have found two sources of differentiation or 'niche partitioning'. On the one hand, we have shown clear differences in tolerance to water shortages, which might explain non-equilibrium coexistence (*sensu* Wiens, 1984 and Illius and O'Connor, 1999). On the other hand, we have shown differences in the degree of plasticity for certain traits that seem to explain those tolerances: the combination of these functional trait responses (from fixed to plastic, and at the performance and mechanistic level), plus the average value for each trait, gives a particular trait configuration that should be considered part of each species' water-use strategy (for light and nutrients, see Busch *et al.*, 2011). Overall, the dominant species in this environment is *Pa. speciosa*, the physiologically most tolerant, and the least abundant one is *B. pictus*, the least tolerant (Soriano, 1956; Golluscio *et al.*, 2005). This suggests that tolerance, in the sense of Grime (1979) and Connell and Slatyer (1977), would be the main mechanism of dominance, as broadly proposed by Tilman's (1988) model, perhaps along with the temporal and spatial variability inherent to arid and semi-arid communities.

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