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

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Abstract

Despite general agreement on the adaptive importance of plasticity, evidence regarding the role of environmental resource availability is scarce, at least in plants. The main hypothesis was the traditional interspecific trade-off hypothesis applied to dryland species; those most tolerant to drought have lower potential growth, and therefore also have the lowest plasticity—here specifying that this only occurs in fitness-related traits. Thus, a derived hypothesis proposes that such stability in fitness is only possible through a higher plasticity in underlying traits (i.e. those associated with response mechanisms). Three conspicuous coexisting perennial grasses from a Patagonian steppe were grown under controlled conditions including four levels of steady-state water availability. The most plastic species along this drought gradient was the most sensitive to drought, whereas the least plastic and overall slower growing one was the most tolerant. This negative relationship between tolerance and plasticity was true for fitness-related traits but was trait-dependent for underlying ones. 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, is a functional one of fixed-vs.-plastic responses.

Keywords
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 each organism 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). "Norm of reaction" type of graphs represent the phenotypic changes (Y axis) due to changes in the environment (X axis), with the slope representing phenotypic plasticity. Weiner (2004) has argued that this definition of plasticity is too broad, and requires a more narrow definition of trait. Although different genotypes may have different plasticity for the same trait, a given genotype may have high plasticity for a trait and low plasticity for another one.

Differences in plasticity for the same trait across environments could be determined by the different selective pressures they would impose. In stable environments, plasticity would not be indispensable since a fixed phenotype would be well adjusted to the most probable condition (Alpert and Simms, 2002; Callaway et al., 2003; Gianoli and González-Tauber, 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 cost would result specially maladaptive in poor environments, whereas in rich environments is less likely to affect fitness (Alpert and Simms, 2002). At least for plants, the experimental evidence on the connection between resource availability in
environment and plasticity is scarce (Alpert and Simms, 2002). This seems to be specially true for water shortages.

In order 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 where 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: Alpert and Simms’ (2002) underlying traits. In other words, stress always has an effect at some level: sometimes is at the ‘macroscopic’ level, affecting fitness, and sometimes only at a more detailed level, modifying underlying traits that allow fitness stability. This distinction would answer Weiner’s (2004) plea for a refinement of the plasticity concept and seems worth 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). A typical depiction of trade-off is the norm of reaction in Figure 1A (largest plot): the solid line shows a typical fast-growing species that is more strongly affected by stress than the typical tolerant species shown by the dashed line. The existence of trade-off is evidenced by the lack of parallelism between lines; and the statistical test of it is the significance of the interaction between stress and species. Here, the different degrees of tolerance between species are revealed by different slopes. The smaller plots in Figure 1A highlight the prediction for the same pair of species under different experimental conditions. The alternative, no-trade-off model is that both species respond similarly to stress, with similar slopes (Figure 1B).
However, if one of them shows a better performance along the entire stress range, it still can be defined as the most tolerant of the pair despite the lack of a trade-off.

In arid and semiarid environments, 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. 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 does not the same as saying that plasticity is always adaptive, for example, a decrease in total plant biomass caused by stress is not. Still, to understand species drought tolerance is necessary to know the plasticity of several water economy-related traits. That species differ between each other in a number of traits is a truism, but what matters for tolerance is their degree of adjustment (i.e. their position in the fixed-plastic continuum).

The western Patagonian steppe has been intensively studied since 1950 as a model of a semiarid system (Soriano, 1956; León et al., 1998). Perennial grasses and shrubs are the dominant functional 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 (67% of net aerial primary productivity; Fernández et al., 1991; Golluscio et al., 2005). Their homogeneity is conspicuous in root distribution (all species have higher root density in the first 30 cm of 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, which suggest a strong niche overlap, begin the question of how to explain coexistence in an environment mainly limited by a single resource (Gausse, 1934). This might be answered by some divergence in other traits, such as root anatomy (Leva et al., 2009) and perhaps function, litter chemistry (Vivanco and Austin, 2005), 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 Mat 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 specially devised for inter-specific tolerance comparisons, controlling for intrinsic and drought-induced differences in plant biomass.

The main goal of this work 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. For this, we evaluated traits at two levels of organisation. 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 (detailed in Methods). The main hypothesis is 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 (Fig. 1A). The secondary hypothesis is that the opposite occurs at the more detailed level: here, the more tolerant species are more plastic in mechanistic traits (those that allow them more constancy in fitness).

**Materials and Methods**

**Plant material**

In order to study the plastic response of perennial grass species to changes in water availability, an experiment was developed under controlled conditions. Those species coexist in West Patagonian District in the Patagonian steppe where the average annual precipitation is 150 mm. This Patagonian community was called “coironal” because of the dominance of “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. y *Senecio filaginoides* DC., and the grasses: *Pappostipa speciosa, Pappostipa humilis* (Cav.) Romansch. and *Poa ligularis* (Golluscio *et al*., 1982; León *et al*., 1998; Golluscio and Oesterheld, 2007). However, the most conspicuous grasses which represent the 80% of the biomass in the grass guild are: *Pappostipa speciosa* (Trin. y Rupr.) Romansch., *Poa ligularis* Nees ap. Steud. y *Bromus pictus* Hook. . Based on their xeromorphic characteristics (Table 1), it is possible that these species have different abilities to tolerate drought.

The seeds used in experiment were collected during the summer of 2008 at the INTA Rio Mayo Experimental Station (lat 45° 41´ S; long 70° 16´W), in a typical coironal community. Mean annual precipitation in the site is 152 ± 44 mm and mainly falls during autumn and winter (Jobbágy *et al*., 1995). The seeds were placed in germination
chambers at 15ºC, with water and in the dark in IFEVA, FAUBA/CONICET. After emergence the seedlings were transplanted to individual plastic pots filled with sand. Plants were irrigated daily with nutrient solution to reach a size of 2 tillers and then, be assigned to one drought treatments.

**Drought device**

Being aware of the potential effects of size/biomass on water stress in potted plants (e.g. Ray and Sinclair, 1998; Passioura, 2002; Fernández, 2010) we used a method specifically devised to avoid the feedback 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 (Figure 2). It was first proposed by Haan and Barfield (1971) and then developed by Snow and Tingey (1985) and used in several studies (e.g. Fernandez 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 porous commercial Styrofoam (Smithers-Oasis) along which the water rises through capillary action to the base of each pot (Figure 2). 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 undergone four levels of drought during 60 days 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 sixteen plastic containers (4 water levels x 4 replicates arranged in blocks). Eighteen pots were placed in each container on top of Styrofoam columns as explained above (18 = 6 pots or subsamples x 3 species, representing the species variability). The total number of pots in the experiment was 288 (18 pots x 4 drought levels x 4 blocks). The four levels of water availability (water table depth) were: 6 cm (control), 13 and 20 cm (intermediate drought), and 27 cm (extreme drought). 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 with plants were filled with sand and fitted at their base with a permeable cloth.

In the glasshouse, maximum PAR (photosynthetically active radiation) at noon was 825 μmol m⁻² s⁻¹, and daily temperatures ranged between 25 and 10 °C. Three tillers of each plant and 3 leaves of each tiller were identified and marked during the experiment (total number of marked tillers was 1,152 and leaves 3,456). 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: aboveground biomass (g), root biomass (g), tiller density (tiller pot⁻¹). These data were collected for each pot and then seven variables were calculated: leaf elongation rate (LER, mm leaf day⁻¹), leaf senescence rate (LSR, mm new yellow tissue day⁻¹), final lamina length
(FLL, mm), tillering (number of new tillers day⁻¹), tiller biomass (g tiller⁻¹), total biomass (g) and shoot/root biomass ratio (g) (see Figure 3).

**Studied traits**

Evaluated traits were included in two different groups (Figure 3). One is made up by traits associated with potential plant performance and correlated with fitness, and were called “performance traits”: **aboveground biomass**, **total biomass** (aboveground and root biomass), **tillering** (number of new tillers appeared by day), and **tiller density** at harvest (Fig. 1). The second group included traits associated with mechanisms of plant adjustment to environmental changes–water changes in this case. There were called “mechanistic traits”: **root biomass**, **shoot/root biomass ratio**, **tiller biomass** (total biomass divided by number of tillers), **final leaf length** (FLL: length of total elongated leaf), **leaf senescence rate** (length of yellow tissue divided by time) and **leaf elongation rate** (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 analysis**

Results were analyzed 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, aboveground biomass and tiller biomass were square root transformed to ensure homogeneity of variance. Interaction between block and treatment was always non-significant. Therefore, normality, homogeneity and block-treatment additivity assumptions were fulfilled. Results were graphically presented as ‘norms of reaction’ (on X axes the environmental gradient: drought; on Y axes
evaluated traits). Norms of reaction for a given genotype are determined by changes in
the phenotype in response to environmental changes. If the norms of reaction of several
species are similar (similar slopes), this means that species have similar plasticity and
that there is no trade-off between performance and tolerance (Sambatti and Caylor,
2007). For clarity, results are shown as graphic ‘reaction norms’, but the corresponding
statistical analyses are summarized in Table 2.

To summarize: on the one hand, plasticity index (IP, sensu Valladares et al.,
2000) was calculated for each species by integrating different evaluated traits. On the
other hand, three average plasticity indexes were evaluated. The first index included
only performance traits, the second index included mechanistic traits and the third one
included all the studied traits.

\[ IP = \frac{(A_{\text{max}} - A_{\text{min}})}{A_{\text{max}}} \]

\( A_{\text{max}} \) is the treatment average with highest values (control) and \( A_{\text{min}} \) the treatment
average with lowest values (extreme drought).

**Results**

The three traits evaluated at the leaf level (LER, LSF, FLL) showed a significant
interaction between drought and species (Table 2, Figure 4): for all the species, values
decreased with increased drought but in different proportion. *Bromus pictus* was the
species with the highest trait values under non-limiting conditions (Figure 4). However,
when drought increased, LER, LSF and FFL decreased to values resembling those of
the other species. Average values of *B. pictus* for these traits were also higher than for
the other two species. Among these, *B. pictus* was the one most affected by drought
(had a higher slope in its norm of reaction; Figure 4), followed by *P. ligularis* and then by *P. speciosa*.

Tillering, and thus tiller density, also showed a significant interaction between drought and species; *P. speciosa* was clearly less affected by drought than the other species (Table 2, Figure 5B). 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, Figure 5A). As happened for the leaf-level traits, *B. pictus* again showed the highest average value for tiller biomass (5 times higher) and *P. 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. Tiller density and tillering showed a different ranking than for the other studied traits: averaging across treatments, the highest values corresponded to *P. ligularis* and the lowest to *B. pictus*. The species *P. speciosa* was similar to *B. pictus* in the control treatment but similar to *P. ligularis* under extreme drought. In addition, *P. 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 aboveground biomass had a significant interaction between drought and species (Table 2, Figure 6A,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. *P. ligularis* showed intermediate values for aboveground biomass while in total biomass showed similar values to those of *P. speciosa*. Shoot/root ratio differed between species and drought treatments but did not have a significant interaction between them (Figure 6D). Root biomass presented marginally significant drought x species interaction (p = 0.07): there were more differences between species than between treatments (Table 2, Figure 6B).
Root biomass of *P. ligularis* showed overall low values and *P. speciosa* always showed the highest ones. For the same trait, a decreasing tendency was found in *B. pictus* when drought increased. Conversely, *P. speciosa* increased root biomass when drought increased.

As happened for most previous traits, *B. pictus* presented the highest average values in total biomass, aboveground biomass and root biomass. *P. 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. 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 the species kept tiller biomass rather constant along the water supply gradient. High plasticity in tiller density seems to be the way to preserve tiller biomass along the gradient.

The plasticity index of each single trait (Figure 7) revealed tiller biomass and root biomass as the most fixed traits. On the other hand, tiller density was the most plastic trait. The behavior of this last trait evidences that the response of the three species under stress conditions is first observed (at the more visible level: performance trait) through the adjustment of tiller density. Regarding mechanistic traits, leaf length showed a high plasticity, with values similar to those of aboveground biomass (a performance trait). At the same time, considering species contribution to plasticity of root biomass (the most fixed trait) the first species were *B. pictus* and *P. speciosa*, species in opposite ends of the gradient. In the case of *B. pictus* drought increase resulted in a decrease of root biomass and, in the case of *P. speciosa* resulted in an
increase. The latter situation is only observed in this case, and can be called positive plasticity.

Unlike mechanistic traits that presented only tendencies in their responses, plasticity indexes for performance traits showed differences between species (Figure 8A,B). In both types of traits, the index of plasticity ranking was the same: *B. pictus* with highest values followed by *P. ligularis* and, in the last place in the ranking, *P. speciosa*. Accordingly, both indexes of plasticity combined into only one index showed the same results (Figure 8C). Based on this combined index, *P. speciosa* was the least plastic species and *B. pictus* was the highest at both types of traits. The specie *B. pictus*, the most plastic one, has a higher plasticity in performance traits in comparison with mechanistic. In turn, *P. speciosa* had similar values in performance and mechanistic traits, and in this last type values included one case of positive plasticity (root biomass).

A performance measure unequivocally related to fitness is the proportion of dead plants for each species and drought level. The overall most plastic species, *B. pictus*, was also the most sensitive to drought: the number of dead plants increased with increased drought. At the other extreme, the least plastic species, *P. speciosa* was the least sensitive to drought: death rates were similar across the entire drought gradient. The proportion of *P. ligularis* dead plants was intermediate (Figure 9).

**Discussion**

As expected from the trade-off model, these three Patagonian species showed different responses to drought for most performance traits (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 all the information available for the species (see Introduction and Table 1), and considering the precautions taken to avoid a positive correlation between plant biomass and water stress (see Drought Device in Methods), this seems to be the first conclusive study. Moreover, previous inter- and intra-specific studies associated with water on C4 perennial grass species have shown a lack of trade-offs (Fernández and Reynolds, 2000; Couso et al., 2010).

The species *B. pictus* turned out to be the most sensitive to drought of the three species. Under close-to-optimal conditions it showed the highest total and aboveground biomass, values that were reduced by more than 50% under stress. A comparable relative reduction was observed for tiller number, with a slope similar to *P. ligularis* and steeper than for *P. speciosa*. Besides, *B. pictus* had the highest potential growth, i.e. the largest potential to capitalize water pulses for improving performance traits, and then would fit into a water-spending, or Grime’s competitive strategy (1979). Therefore, the slow growing species showed the lowest effect of drought aboveground and total biomass in comparison with the two fast growing species (similar to *Miscanthus* grass genotypes; Clifton-Brown and Lewandowski, 2000). This is consistent with *B. pictus* senescence rate, revealing the high tissue turnover associated with a high RGRmax (Reich, 1998). Interestingly, senescence was plastic in a counterintuitive direction: higher leaf longevity under drought. This would permit to stabilize carbon gain in the face of a low biomass production, as for example has been observed for *Cryptantha flava*, other herbaceous perennial of arid zones (Casper et al., 2001) and for trees (Leuzinger et al., 2005).
At the other end, *P. speciosa* was the most tolerant species, with almost constant total biomass across the water availability range (slope undistinguishable from zero; \( p=0.929 \)). Aboveground biomass and tiller number did decrease, but with gentle slopes. All this would make *P. speciosa* fit a water-saving, or Grime’s (1979) stress-tolerant strategy. In the case of *P. ligularis* biomass, both for average values and in response to stress, was intermediate between that of the other species. However, its tillering rate and density experienced a highest reduction under stress than for the other two species. This was coincident with *P. 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 C₃ perennial grasses of this Patagonian steppe was, thus: *P. speciosa* > *P. ligularis* > *B. pictus*. The overall plasticity ranking, as shown by Figure 8C, was the opposite one, with the most tolerant species being the least plastic one in performance traits (Figure 1A), 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 to response mechanisms. The answer, given by the coincident rankings for performance traits, mechanistic traits, and therefore overall plasticity is a clear “no”. On the contrary, plants responding more to changes in their environment at the visible scales are having parallel and less apparent comparable changes occurring at the same time and likely explaining such response. If it is a lack of plasticity at all levels what characterizes tolerant species, this begs the question of whether possessing rather fixed traits is the most adaptive way to thrive in dry environments. The answer is not straightforward, or at least requires to specify the type
of trait, because the high tolerance (and generally low plasticity) of *P. speciosa*, seems to be explained by the high plasticity of the couple of mechanistic traits describing allocation: root biomass and shoot/root biomass.

Going back to the general question of coexistence of plant species, particularly of these species belonging to the same functional group and allegedly competing mostly 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, 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 (light and nutrient see Busch *et al.*, 2011). Overall, the dominant species in this environment is *P. speciosa*, the physiologically most tolerant one, 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 semiarid communities.

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1 **Tables**

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

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patagonian grass species</th>
<th>Source</th>
</tr>
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<tbody>
<tr>
<td></td>
<td><em>B. pictus</em></td>
<td><em>P. ligularis</em></td>
</tr>
<tr>
<td>N (%)</td>
<td>0.61</td>
<td>0.41</td>
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<td>lignin (%)</td>
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<td>8.12</td>
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<td>lignin/N</td>
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<td>0.52</td>
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<tr>
<td>C:N leaf</td>
<td>33.9</td>
<td>61.9</td>
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<tr>
<td>cover (%)</td>
<td>1.28</td>
<td>3.56</td>
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<tr>
<td>frequency (%)</td>
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<td>98</td>
</tr>
<tr>
<td>palatability</td>
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<td>intermediate</td>
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**XEROMORPHISM**  smaller  intermediate  higher
Table 2: ANOVA results. Response of 3 Patagonian grass species to 4 levels of drought and their interaction. Significance levels: *p < 0.05; ** p < 0.001.

<table>
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<tr>
<th>Level</th>
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<th>Drought effect</th>
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<td></td>
<td>F</td>
<td>p</td>
<td>F</td>
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<td>LEAF</td>
<td>Leaf elongation rate</td>
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<td>Leaf senescence rate</td>
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<td>20.55</td>
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<td></td>
<td>Final leaf length</td>
<td>14.57</td>
<td>&lt;0.01**</td>
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<td>TILLER</td>
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<td>Tillering</td>
<td>29.5</td>
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<td>Tiller density</td>
<td>32.07</td>
<td>&lt;0.01**</td>
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<td>WHOLE</td>
<td>Aboveground biomass</td>
<td>12.39</td>
<td>&lt;0.01**</td>
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<td>PLANT</td>
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<td></td>
<td>Total biomass</td>
<td>6.2</td>
<td>0.014**</td>
<td>63.25</td>
</tr>
<tr>
<td></td>
<td>Shoot/root biomass</td>
<td>15.04</td>
<td>&lt;0.01**</td>
<td>26.17</td>
</tr>
</tbody>
</table>
Figure legends

Figure 1: Theoretically possible norms of reaction showing the relationships between stress tolerance and plasticity. Solid lines represent the species most sensitive to stress; dashed lines the most stress tolerant. (A) Shows the trade-off case, as perceived under different experimental conditions: larger figure shows a wide environmental gradient and smaller figures possible outcomes from narrower sets of conditions at both sides of the same gradient (statistically, a significant species x stress interaction is predicted for all 3 cases). (B) Shows the no-trade-off, case: a better performance in one side of the gradient does not imply a worse one in the other; smaller figures show different possible outcomes, all of which have statistically significant species and stress main effects (and no interaction).

Figure 2: Drought device, based on sub-irrigation technique of Snow and Tingey (1985). C: plastic container; F: hygroscopic foam (porous commercial Styrofoam); N: nutrient solution; a,b,c: sand-filled pots with a permeable base (polyester fabric); z water-table depth (level of water availability). Redrawn from Fernández and Reynolds 2000, Oecologia 123:90-98.

Figure 3: 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 included “performance traits”, traits directly associated with fitness (in frames) and the “mechanistic traits” associated with mechanical response when the plants are subjected to drought conditions (without frames).

Figure 4: Norms of reaction at the leaf level. Graphs represent the response to drought level, means ± SE (control, two intermediate drought levels and extreme drought) for
three Patagonian grasses: *Bromus pictus*, *Poa ligularis* and *Pappostipa speciosa*. (A)

Leaf elongation rate: LER. (B) Leaf senescence rate: LSR. (C) Final leaf length: FLL.

Figure 5: Norms of reaction at the tiller level. Graphs represent the response to drought level, means ± SE (control, two intermediate drought levels and extreme drought) for three Patagonian grasses: *Bromus pictus*, *Poa ligularis* and *Pappostipa speciosa*. (A) Tiller biomass. (B) Tillering and tiller density.

Figure 6: Norms of reaction at whole plant level. Graphs represent the response to drought level, means ± SE (control, two intermediate drought levels and extreme drought) for three Patagonian grasses: *Bromus pictus*, *Poa ligularis* and *Pappostipa speciosa*. (A) Aboveground biomass. (B) Root biomass. (C) Total biomass. (D) Shoot/root biomass.

Figure 7: Plasticity index \[ IP = (M_{\text{max}} - M_{\text{min}}) / M_{\text{max}}; \text{sensu Valladares et al. 2000} \] for each trait for each Patagonian grass species: *Bromus pictus*, *Poa ligularis* and *Pappostipa speciosa*. Left quadrant (mechanistic traits): tiller biomass, root biomass, shoot/root biomass, leaf elongation rate (LER) and final leaf length (FLL). Right quadrant (performance traits): total biomass, aboveground biomass, tillering (same values of tiller density).

Figure 8: Plasticity index \[ IP = (M_{\text{max}} - M_{\text{min}}) / M_{\text{max}}; \text{sensu Valladares et al. 2000} \] for each trait for each Patagonian grass species (Bp: *Bromus pictus*, Pl: *Poa ligularis* and Ps: *Pappostipa speciosa*). (A) Plasticity for performance traits: total biomass, aboveground 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 \( p = 0.003 \).

Figure 9: Proportion of dead plants for each Patagonian grass species \((Bromus pictus, Poa ligularis\) and \(Pappostipa speciosa\)) at 4 different drought levels (here increasing upwards within each species) in the complete experimental period \( p = 0.008 \).
**STRESS TOLERANCE**

**A**

- **PLASTICITY trade-off**

- **B**

  - **PLASTICITY NO trade-off**