



Phenotypic plasticity of morpho-chemical traits of perennial grasses from contrasting environments of arid Patagonia



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ABSTRACT

We hypothesized that the combined effect of aridity and relative shrub cover could lead to differences in phenotypic plasticity among perennial grass populations opposed to those expected by aridity. We selected two populations of *Festuca pallescens* and two of *Poa ligularis* from contrasting environments across a gradient of aridity and relative shrub cover. We collected 10–20 bunches of each population. A half of each was herborized (*in situ* set) and the other was transplanted in pots and maintained at soil field capacity during one year (common growing conditions set). We assessed morpho-chemical traits of bunches of each set and calculated the phenotypic plasticity index of each trait as the change in the trait expression between *in situ* and common growing conditions. Both species showed the largest phenotypic plasticity at sites that represent mid values of aridity and relative shrub cover for Patagonian ecosystems. *P. ligularis* showed larger phenotypic plasticity than *F. pallescens*. According to our hypothesis *F. pallescens* showed higher phenotypic plasticity at the mid than at the wet site while the reverse occurred with *P. ligularis*. We concluded that unpredictable environments could lead to increased phenotypic plasticity of traits. This idea should be further investigated.

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1. Introduction

Plant traits vary widely across regional and global climatic gradients (Austin and Sala, 2002; Wright et al., 2004; Bertiller et al., 2006). Several studies identified the main trade-offs characterizing plant performance in a wide range of habitats. Across these gradients, fast growing plants from resource-rich sites are mostly associated with high phenotypic plasticity while low phenotypic plasticity would prevail in slow-growing plants from resource-poor habitats (Garnier, 1998; Cornelissen et al., 1999; Aerts and Chapin, 2000; Fonseca et al., 2000; Wright and Westoby, 2002; Wright et al., 2002, 2004; Reich and Oleksyn, 2004; Roche et al., 2004; Bertiller et al., 2005). However, these relationships are not clearly defined in more constrained gradients such those occurring within arid environments (Moreno and Bertiller, 2012).

Arid ecosystems are characterized by scarce, highly variable, and erratic precipitation (Noy Meir, 1973). Shrubs and perennial grasses are the dominant plants in most arid ecosystems of the world (Noy Meir, 1973; Aguiar and Sala, 1994; Adler et al., 2004; Armas et al.,

2008) and some studies showed that shrub cover is positively correlated to aridity across regional gradients (Bertiller et al., 2005, 2006). Perennial grasses and shrubs have different mechanisms of resistance to water shortage. Perennial grasses are usually drought-avoiding species with more mesophytic traits and higher phenotypic plasticity than shrubs (Coughenour et al., 1984, 1985; Busso and Richards, 1993; Craine et al., 2002; Grassein et al., 2010; Pazos et al., 2010). In contrast, shrubs are drought-tolerant plants having usually xerophytic traits and well-developed chemical and structural defenses against water shortage and herbivores (Bertiller et al., 1991; Ivanov et al., 2008; Campanella and Bertiller, 2009). Due to these characteristics, shrubs may facilitate the establishment and growth of perennial grasses by creating ameliorated microenvironments around them or protecting herbaceous plants from herbivores (Aguiar and Sala, 1994, 1999; Bertiller et al., 2002; Bertiller and Ares, 2008; Lopez et al., 2009).

Perennial grass species display high morphological variability across both regional and local arid-semiarid gradients and there is evidence that this trait variability may result from complex interactions between climatic gradients and local neighborhoods (Correa, 1978; Fernandez et al., 2004; Bertiller et al., 2006; Moreno and Bertiller, 2012). It may be expected higher plasticity in

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perennial grasses from environments with higher and predictable resource levels than in resource-poor environments (Lambers et al., 2000). In this sense, fixed traits could be thought as the most adaptive way to cope with aridity. However, Couso and Fernandez (2012) reported increasing phenotypic plasticity for fitness-related traits (those related to growth, reproduction and survival) of perennial grasses and decreasing capacity to tolerate drought with decreasing aridity. However, these authors also found trait-dependent responses for other traits such as mechanistic traits describing allocation (root biomass and shoot/root biomass). In contrast, Moreno and Bertiller (2012) showed that the variation of traits of perennial grasses related to drought tolerance across aridity gradients may be opposite to that expected by the effect of aridity (i.e. enhanced mesophytism, reduced chemical defenses at the driest sites) reflecting escape from drought and/or herbivores in the mild or protected neighborhoods induced by local shrub arrangements. This might indicate that both aridity and shrubby neighborhoods could affect oppositely perennial grass traits in arid environments and these trait variations may be either attributed to phenotypic plasticity (Grassein et al., 2010; Couso and Fernandez, 2012) or to different genotypes with fixed phenotypes (Oyarzabal et al., 2008). Accordingly to these results, we hypothesized that the combined effect of aridity and relative shrub cover could lead to differences in phenotypic plasticity among perennial grass populations opposed to those expected by aridity. To test this hypothesis, we evaluated changes in morpho-chemical traits between *in situ* and common growing conditions to assess phenotypic plasticity of perennial grass populations of *Poa ligularis* and *Festuca palllescens* from sites with contrasting aridity and relative shrub cover.

2. Materials and methods

2.1. Study species and sites

P. ligularis Nees ap. Steudel and *F. palllescens* (St-Yves) Parodi are two perennial grasses with a wide geographical distribution in Patagonia and high morphological variability across the environments where they grow. *F. palllescens* is characteristic of semiarid ecosystems and the morphology of this species may vary from tall bunches with long leaves to low bunches with short leaves (Correa, 1978). This variation was observed across both regional and local environmental gradients (Fernandez et al., 2004; Moreno and Bertiller, 2012). The phenology of this species varies among sites with different temperature and/or water regime and this variation was maintained when growing in common gardens (Ares et al., 1990). *P. ligularis* is a perennial grass characteristic of arid ecosystems displaying high genetic (Hunziker, 1978) and phenotypic variability (Correa, 1978; Giussani et al., 1996; Giussani, 2000). Moreno and Bertiller (2012) reported changes in leaf traits of *P. ligularis* and *F. palllescens* with increasing aridity as well as local leaf trait changes induced by the vicinity to shrubs.

We selected two populations of each perennial grass species (*P. ligularis* and *F. palllescens*) from sites with contrasting aridity index (AI) and relative shrub cover (RSC). The selected sites were PLdry (AI: 7.2, RSC: 92%), PLmid (AI: 3.9, RSC: 31%) for *P. ligularis* and FPmid (AI: 3.68, RSC: 23%), FPwet (AI: 1.07, RSC: 0.01%) for *F. palllescens* at the dry and wet site, respectively. These sites are near the driest and the wettest extremes of the distribution areas of each species encompassing those of the extra-Andean Patagonian ecosystems (Bertiller et al., 2006). The RSC (shrub cover as percent of total cover) and the AI, calculated from mean annual temperature (MAT) and mean annual precipitation (MAP), of each site were taken from Carrera and Bertiller (2010) and Moreno et al. (2010). High AI-values indicate high aridity conditions. These sites differ in

the amount and in the inter-annual variation of annual precipitation. The gamma parameter, characterizing the inter-annual variability of the precipitation (Barros and Rivero, 1982), also varied among *P. ligularis* sites: Sierra Chata: 3.90, Pampa del Castillo: 4.35 and *F. palllescens* sites: Gastre: 4.2, Ea La Pepita: 10. A large gamma parameter indicates low inter-annual variation of precipitation, thus precipitation is more variable, more erratic, and less predictable in the sites with the highest aridity index than in the wettest extreme of the aridity gradient (Table 1).

2.2. Material collection, common growing conditions and morpho-chemical traits

Both species are perennial bunch (caespitose) grasses (Correa, 1978) and each bunch is a compact spatial arrangement of clonal tillers (genet) (Briske and Derner, 1998). We randomly collected between 10 and 20 bunches of each species at each site during the period from end of vegetative growth to early reproductive growth (November–December 2007). A half of the tillers of each bunch were herborized to assess morpho-chemical traits *in situ*, and the other half were transplanted in pots (1.5 l) to assess morpho-chemical traits in a common garden. The pots were filled with a soil mixture in equal proportion of soils of wet sites (high fertility: N: $1.35 \pm 0.05 \text{ mg g}^{-1}$, C: $12.42 \pm 1.03 \text{ mg g}^{-1}$), and dry sites (low fertility: N: $0.44 \pm 0.02 \text{ mg g}^{-1}$, C: $5.59 \pm 0.15 \text{ mg g}^{-1}$). Subsequently, the pots were placed in a partially shaded place in an experimental site (CENPAT, Puerto Madryn- Chubut: $42^{\circ}49'46'' \text{ S}$, $65^{\circ}04'56'' \text{ W}$) in December 2007. The use of a soil mixture in the pots of the common garden and shading allowed homogenizing the substrate and reducing the atmospheric demand in order to reach “optimal” common growing conditions but could eventually introduce some confounding effect of nutrients and shading on the experiment. All the pots received weekly irrigation to field capacity (20%) during 12 months. Soil moisture was controlled with a TDR IMKO sensor. The mean annual precipitation during the study was 100.1 mm, and the mean annual temperature was 14.3° C (Laboratory de Climatology of the National Patagonian Center-CENPAT-CONICET, www.cenpat.edu.ar). The common garden conditions represented situations with large periods of continuous and high soil humidity (20% throughout the one-year study period) more favorable than those prevailing *in situ* for the four study perennial grass populations. Although, the yearly water balance in the wet extreme is nearly at equilibrium between precipitation and evapotranspiration, precipitation is concentrated in the cold seasons (autumn and winter) and soils in the rooting depth have water contents lower than 7% during summer (Coronato and Bertiller, 1996). Similarly, soil water content in the rooting depth in the field at the dry extreme of the gradient in summer is lower than 6% (Coronato and Bertiller, 1997) thus achieving contrasting environmental conditions in the field compared to the common garden. Aboveground parts of plants of each pot were harvested in December 2008.

We measured morphological and chemical traits on homogeneous tillers (number of leaves, tiller hierarchy) collected from bunches of each species and site grown *in situ* and under common garden conditions. Herborized plants were rehydrated before measurements. Tiller height ($n = 3$ per bunch) was measured in vegetative tillers from the base up to the top of the longest leaf. Length, width, and area of blades were measured on digitized images of the youngest full expanded green leaf of each tiller ($n = 5$ tillers per bunch). After that, blades were dried at 60° C for 48 h and weighed to estimate the dry mass. The specific blade area (SBA) of the youngest full expanded green leaf of the tiller was calculated as blade area/blade dry mass ($n = 5$ tillers per bunch). We used the blades of the remnant totally full expanded young green leaves

Table 1
Location, Phytogeographical Province (P: Patagonian, M: Monte) and Phytogeographical District (S: Subandean, O: Occidental, SJG: San Jorge Gulf), aridity index (AI), mean annual temperature (MAT), mean annual precipitation (MAP), gamma parameter of intra-annual variation of precipitation (G), and relative shrub cover (SRC) of sites of each population of *Festuca pallelescens* and *Poa ligularis*. Aridity index (AI) = PET/MAP, where PET (mean annual potential evapotranspiration) = 69.4 * MAT (UNESCO, 1979; Le Houérou, 1990). High AI values indicate high aridity. Data on AI, MAT, MAP were taken from Carrera and Bertiller (2010) and Moreno et al. (2010). Data on gamma parameter were taken from Barros and Rivero, 1982. Data on SRC were taken from Moreno and Bertiller (2012).

Species (%)	Site	Site location	Phytogeographic province-district	MAT	MAP	G	AI	SRC
<i>Festuca pallelescens</i>	FPwet	44°58'47.4" S, 71°17'00.0" W	P-S	7.68	500	10	1.07	0.01
	FPmid	42°23'31.0" S, 68°56'41.9" W	P-O	7.95	150	4.2	3.68	23
<i>Poa ligularis</i>	PLmid	45°41'11.6" S, 67°53'26.6" W	P-SJG	8.58	150	4.3	3.97	31
	PLdry	42°45'42.5" S, 66°01'02.5" W	M	13.16	125	3.9	7.31	92

without signs of deterioration (uniformity in the coloration and absence of damage by herbivores or pathogens) of each bunch dried at 60 °C for 48 h to assess N concentration by semi-micro Kjeldahl, C concentration by ashing at 550 °C (Schlesinger and Hasey, 1981), total soluble phenolics by the Folin Ciocalteu method using 50% methanol as extract solution and tannic acid as standard (Waterman and Mole, 1994), and lignin by the van Soest method (van Soest, 1963). We selected these traits since they are directly related to plant processes such as photosynthesis (C), growth (N) and defensive mechanisms (lignin and soluble phenolics).

2.3. Statistical analysis and calculation of the phenotypic plasticity index (PPI)

The significance of the differences in morpho-chemical traits between field (*in situ*), and common garden conditions of each species and site was quantified by ANOVA after testing the assumptions of this analysis. The level of significance was $p \leq 0.05$ throughout the study. We quantified the change in the expression of each morpho-chemical trait using the phenotypic plasticity index (PPI), suggested for Valladares et al. (2000), as:

$$PPI = \frac{(\text{maximum mean} - \text{minimum mean})}{\text{maximum mean of each trait value}}$$

The PPI was only calculated for those traits differing significantly between field and common garden conditions. The value of the PPI varies between 0 and 1, where 1 is the maximum value of change in the expression of a trait. We performed cluster analysis and one way ANOVA to compare the PPI across traits. All statistics analyses were performed with the statistic package SPSS 7.5 for Windows (Norusis, 1997).

3. Results

3.1. Variation between *in situ* and common growth conditions of the morpho-chemical traits of perennial grasses

Tiller height, blade weight, and blade area showed significant differences between growth conditions in the FPmid and in the PLmid populations (Fig. 1a–c). Blade length varied significantly between growth conditions in both populations of *F. pallelescens* (FPwet and FPmid) and in the PLmid population and also (Fig. 1d). Blade width only varied between growing conditions in the FPmid population (Fig. 1e). All these traits exhibited larger values in bunches growing under common conditions than those growing *in situ*. The specific blade area did not significantly vary between growing conditions in populations of both species (Fig. 1f).

The N concentration in blades of populations of both species varied between growing conditions and it was higher in bunches growing in common conditions than *in situ* (Fig 2a). C in blades showed differences between growing conditions in the FPmid

population (C in blades *in situ* < C in blades in common conditions) and in the PLmid population (C in blades *in situ* > C in blades in common conditions) (Fig. 2b, Table 2b). Total soluble phenols in blades did not significantly vary between growing conditions in populations of both species (Fig. 2c, Table 2b). Lignin in blades varied between growth conditions only in the populations growing in the respective driest site of each species. In both cases, bunches growing at the common conditions exhibited larger values of lignin than those growing *in situ*.

3.2. Phenotypic plasticity index

We found significant phenotypic plasticity indexes (assessed by significant differences between *in situ* and common conditions) mostly at mid sites on the gradient (Figs. 1 and 2). Cluster analysis resulted in five traits-site groups which differed significantly among them ($F_{4, 17} = 182.4, P < 0.01$). PPI values varied from 0.03 to 0.71. *P. ligularis* showed the highest PPI of tiller height, blade length, blade weight, and blade area at mid sites on the gradient (Group I, Fig. 3). *F. pallelescens* showed the highest PPI values of blade length, blade area, and blade also at mid sites of the gradient (Group III, Fig. 3), although these values were lower than those of *P. ligularis*. The PPI of N in blades was higher in FPwet, FPmid and PLdry (0.58, 0.52, and 0.53, respectively, Group II, Fig. 3) than in PLmid (0.18, Group IV). C concentration in blades showed the lowest significant plastic response in FPmid and PLmid (Group V, Fig. 3). Lignin in blades showed significant a plastic response in PLdry and FPmid being this response higher in *P. ligularis* than in *F. pallelescens* (Group III and Group IV, respectively).

4. Discussion

Trait variation between growing conditions at wet and the dry extremes of the whole gradient was very low. At the wet site, relative shrub cover is the lowest, and precipitation is near the annual evaporative demand and less erratic than in the dry site (Noy-Meir, 1973; Barros and Rivero, 1982; Ares et al., 1990). On the other hand, the dry site has the highest relative shrub cover and precipitation is highly erratic and many fold lower than the annual evaporative demand. Under these conditions, dense shrub canopies (92% of relative shrub cover) may protect herbaceous plants from herbivores due to their strong physical and chemical defenses and may create ameliorated and more predictable microenvironments associated with their canopies (Bertiller et al., 2002; Abule et al., 2005; Lopez et al., 2009; Moreno and Bertiller, 2012). This is consistent with more mesophytic traits (i.e. higher tillers, larger blade areas and longer blades) in the dry site than those at mid sites, reinforcing the role of shrubs in the creation of ameliorated and more predictable microenvironments associated with their canopies (Bertiller et al., 2002; Abule et al., 2005; Lopez et al., 2009; Moreno and Bertiller, 2012). Therefore, contrarily to general expectations (Garnier, 1998; Valladares et al., 2006; Couso and

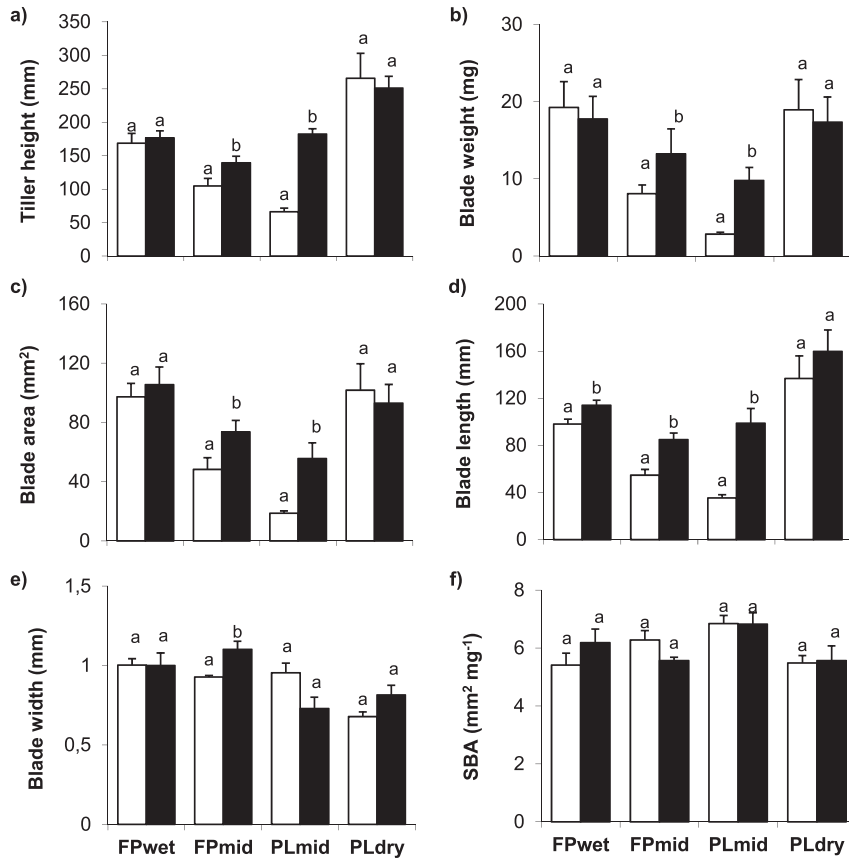


Fig. 1. Mean \pm one standard error of a) tiller height, b) blade weight, c) blade area, d) blade length, e) blade width, and f) specific blade area (SBA) of FPwet, FPmid, PLmid, and PLdry growing *in situ* (white bar) and in common conditions (black bar) at each collection site. RSC: relative shrub cover, AI: aridity index. Different small case letters indicate significant differences ($p < 0.05$) between growing conditions.

Fernandez, 2012), perennial grasses displayed lower phenotypic plasticity of morphological traits under more predictable and stable environmental conditions for growth (wet and dry sites) than at mid sites. However, we found large phenotypic plasticity of N

concentration in blades of both species across the whole gradient. This is a particularly important feature since N is a limiting nutrient in most terrestrial ecosystems and it is directly involved in plant nutrition, plant growth, organic matter turnover, and nutrient

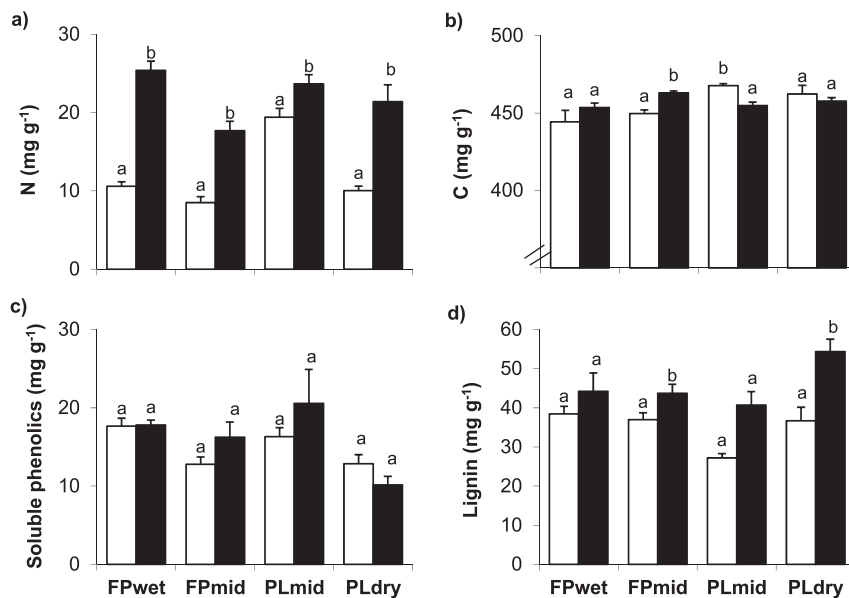


Fig. 2. Mean \pm one standard error of a) nitrogen concentration, b) carbon concentration, c) soluble phenolics concentration, and d) lignin concentration of FPwet, FPmid, PLmid, and PLdry growing *in situ* (white bars) and in common conditions (black bars) at each collection site. RSC: relative shrub cover, AI: aridity index. Different small case letters indicate significant differences ($p < 0.05$) between growing conditions.

Table 2
Results of ANOVA comparing a) morphological and b) chemical traits, of FPwet, FPmid, PLmid, and PLdry between growing conditions (*in situ* and common conditions). RSC: relative shrub cover (%) AI: Aridity Index.

Species	<i>Festuca pallescens</i>								<i>Poa ligularis</i>							
	FPwet AI: 1.1; RSC 0.01%				FPmid AI: 3.6; RSC 23%				PLmid AI: 3.9; RSC 31%				PLdry AI: 7.3; RSC 92%			
	Df1	Df2	F	P	Df1	Df2	F	P	Df1	Df2	F	P	Df1	Df2	F	P
Traits																
a) Morphological																
Tiller height	1	12	0.12	ns	1	12	4.87	0.04	1	12	133.41	<0.001	1	12	0.07	ns
Blade weight	1	11	0.07	ns	1	12	8.74	0.01	1	12	41.31	<0.001	1	12	0.06	ns
Blade area	1	11	0.27	ns	1	12	4.90	0.04	1	12	38.70	<0.001	1	12	0.09	ns
Blade length	1	12	5.4	0.04	1	12	16.61	0.002	1	12	54.71	<0.001	1	12	0.51	ns
Blade width	1	11	0.002	ns	1	12	18.3	0.002	1	12	4.41	ns	1	12	4.01	ns
Specific blade area	1	11	1.25	ns	1	12	3.31	ns	1	12	0.001	ns	1	12	0.03	ns
b) Chemical																
N in blades	1	12	6.83	<0.001	1	16	34.75	<0.001	1	18	161.78	0.02	1	15	36.31	<0.001
C in blades	1	12	0.80	ns	1	14	29.89	<0.001	1	15	24.73	<0.001	1	14	0.39	ns
Total soluble phenols in blades	1	10	0.006	ns	1	10	4.31	ns	1	7	0.45	ns	1	8	4.16	ns
Lignin in blades	1	9	4.00	ns	1	14	5.84	0.03	1	6	4.57	ns	1	8	17.41	0.003

Significant differences are indicated in bold.

cycling (Aerts and Chapin, 2000; Westoby et al., 2002). Moreover phenotypic plasticity in N acquisition indicates that both species are able to capture more N under high predictable climatic conditions (common conditions) with large periods of continuous high soil humidity and probably high microbial activity and N release (Mazzarino et al., 1998). This in turn highlights that water is a more limiting factor than light or nutrient availability. Furthermore, the population of *P. ligularis* from the dry site also showed large phenotypic plasticity in a defense related trait (lignin concentration) indicating that under predictable water conditions this population is able to increase structural chemical components of blades. This could be associated with a strategy of *P. ligularis* populations from the dry site to rapidly accumulate carbon-based secondary metabolites in these environments where N is more limiting than water for growth (Austin and Vitousek, 1998; Bertiller et al., 2006). Thus, accumulation of N and lignin under favorable conditions could help to maintain adequate levels of nutritional

and defensive compounds avoiding high costs of nutrient acquisition and chemical defense production during adverse periods (Crawley, 1998; Fitter, 1998; Garnier, 1998).

In contrast to extreme environmental conditions, populations of both species from mid sites displayed large phenotypic plasticity in traits. In the case of *F. pallescens*, our findings support our hypothesis that local environments induced by shrub canopies could be related to differences in phenotypic plasticity of morphological and chemical traits of perennial grasses opposed to those expected by aridity. However, in the case of *P. ligularis* phenotypic plasticity of morphological traits was higher at mid than at dry sites, as expected by aridity.

In conclusion, our results could indicate that populations of both perennial grass species from sites with variable and unpredictable environmental conditions may display opportunistic vegetative growth responses and enhanced N acquisition (large phenotypic plasticity) under conditions of high resource availability as reported

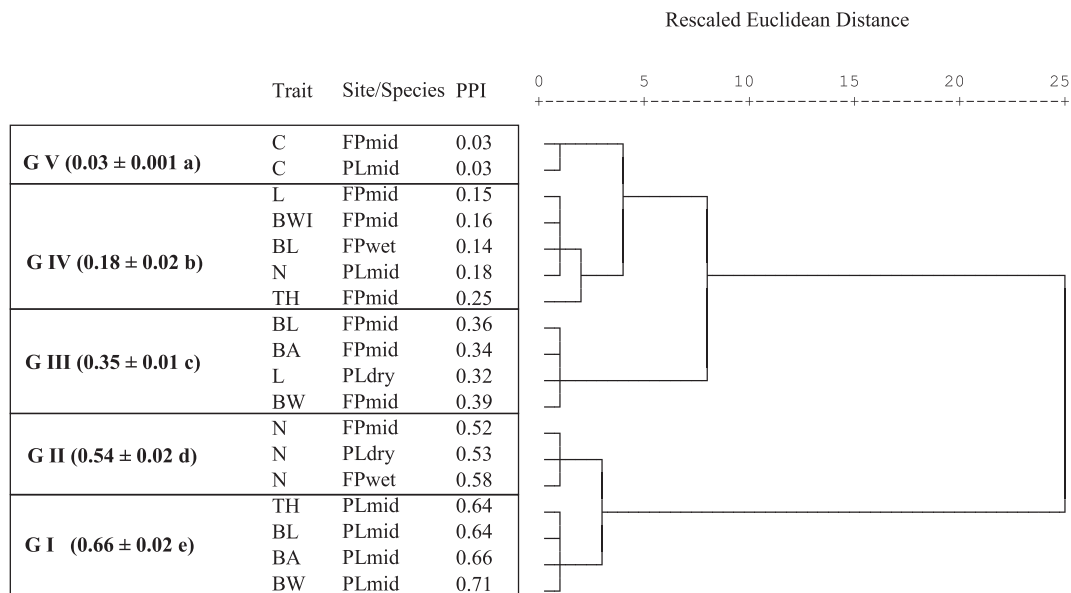


Fig. 3. Groups of species traits by site (G I to G V) resulting from cluster analysis based on the phenotypic plasticity index (PPI). In parentheses mean PPI ± 1 standard error. Morphological traits: tiller height (TH), blade length (BL), blade width (BWI), blade area (BA), and blade weight (BW). Chemical traits: nitrogen concentration (N), carbon concentration (C), and lignin concentration (L). The PPI was only calculated for those traits differing significantly between field and common garden conditions (Figs. 1 and 2). Lower case letters indicate significant differences ($P \leq 0.01$) among mean PPI of groups.

for perennial grasses of other ecosystems (Danquah, 2010; Grassein et al., 2010). Moreover, *P. ligularis* occupying dry and more unpredictable sites showed higher phenotypic plasticity compared to *F. palleescens*. Accordingly, our findings could highlight that limiting and variable environments could promote not only species diversity (Chesson et al., 2004) but also functional diversity through phenotypic plasticity for a single species trait depending on resource availability. However it should be noted that these results could be influenced by the limited number of species and range of common environmental conditions that we explored in our study and the lack of replication in different years. In this sense, further research would be needed including more species and experiments with more than one year of duration, to assess the eventual variation of the phenotypic plasticity among years with different precipitation and temperature conditions.

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