Trait differences between grass species along a climatic gradient in South and North America

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Abstract

Question: Are trait differences between grasses along a gradient related to climatic variables and/or photosynthetic pathway?

Location: Temperate grassland areas of South and North America.

Methods: In a common garden experiment, we cultivated C_3 and C_4 grasses from grasslands under different climatic conditions, and we measured a set of 12 plant traits related to size and resource capture and utilization. We described (1) interspecific plant trait differences along a climatic gradient defined by the precipitation and temperature at the location where each species is dominant and (2) the association between those plant trait differences and the photosynthetic pathway of the species.

Results: Trait differences between grasses were related to the precipitation at the area where each species is dominant, and to the photosynthetic pathway of the species. Leaf length, leaf width, plant height, leaf area per tiller, specific leaf area, leaf $\delta^{13}C$ ratio, and nitrogen resorption efficiency increased while leaf dry matter content and nitrogen concentration in senesced leaves decreased as precipitation increased. A proportion of these changes along the gradient was related to the photosynthetic pathway because dominant grass species in cold areas with low precipitation are mainly C_3 and those from warm and wet areas are C_4.

Conclusions: A previous worldwide analysis showed that traits of graminoid species measured *in situ* changed slightly along climatic gradients (< 10% variance explained). In contrast, under a common environment we observed that (1) grass traits changed strongly along a climatic gradient (30-85% variance explained) and, (2) a proportion of those changes were related to the association between photosynthetic pathway of the species and precipitation.

Keywords: Dominant grass; Plant trait; Precipitation; Temperate grassland; Temperature.

Nomenclature: Cabrera & Zardini (1978); Correa (1971-1988); Anon. (2005).

Abbreviations: MAP = Mean annual precipitation; MAT = Mean annual temperature.

Introduction

Understanding how plant morphology and physiology change under different environmental conditions could reflect the existence of trends in traits specialization and provide indirect evidence on the variation of ecosystem level processes. For example, differences between plants related to leaf size, area, and height along an environmental gradient suggest an increase of light competition. Similarly, changes on nutrient conservation strategies along a gradient could be indirect evidence of variations in resource limitations. In that way, these trait changes along gradients are potentially useful predictors of ecosystem functioning. The challenge is to describe plant traits from different areas minimizing the site effects, which may be achieved by studying trait variations under a common environment (e.g. Coughenour et al. 1985).

Descriptions of plant trait variations along gradients are common, but we identified a particular gap of knowledge: the description, under a common environment, of plant trait variation along broad environmental gradients. A number of studies have concentrated on variations of woody species traits, suggesting a predictable set of scaling relationships themselves (Reich et al. 1999; Reich et al. 1998; Wright et al. 2001). In addition, other studies have explored the variations of these woody vegetation traits along environmental gradients. For example, mature canopy height, leaf width, lamina thickness, and specific leaf area increased along gradients of both water and nutrient availability (Cunningham et al. 1999; Fonseca et al. 2000). Since plant traits were measured *in situ*, a proportion of the plant trait variation along the gradient could be due to interspecific differences and/or site effects.

Even though herbaceous plants also have received attention, the studies have consisted of either single-site surveys or comparisons along climatic gradients measuring traits *in situ*. For example, Craine et al. (2001,

2002) described root and leaf traits along environmental gradients but the species were from a common area, the grasslands of central Minnesota, USA. Certainly the most extensive and profound survey of this sort was the one carried out by Wright and collaborators at a global scale along wide climatic gradients, but in this case most of the plant trait measurements were *in situ* (Wright et al. 2004). Previous descriptions of graminoid traits along a precipitation gradient were made by Coughenour et al. (1984, 1985) for a small species group. Thus, we scarcely know how grass traits vary along broad environmental gradients. Specifically, we do not know if there are different grass trait variations along a gradient independently from the site effect.

Interestingly, two features of grass species offer a unique opportunity for the description of plant trait variations along environmental gradients. First, grass species are dominant in temperate grassland areas covering an extensive gradient, where precipitation (150-1200 $mm.yr^{-1}$) and temperature (0-25ºC) are positively correlated (Lauenroth 1979; Sala 2001). Second, both C_3 and C_4 photosynthetic pathways are common within the *Poaceae* and many plant traits are clearly associated to the photosynthetic syndrome. Previous papers have shown that C_3 and C_4 species differ in a number of structural and functional traits, including leaf and root anatomy, quantum yield, and carbon gain per unit mass (Craine et al. 2001, 2002; Dengler et al. 1994; Ehleringer 1978). Therefore, both mechanisms have differential advantages depending on the specific environment. As a result, the dominance, cover, and productivity of each photosynthetic pathway type change along environmental gradients and determine that dominant grass species in cold areas with low precipitation are mainly C_3 and those from warm and wet areas are C_4 (Cabido et al. 1997; Cavagnaro 1988; Ehleringer 1978; Epstein et al. 1997, 1998; Paruelo & Lauenroth 1996).

The objectives of our study were: (1) to describe interspecific grass trait differences along a climatic gradient defined by the precipitation and temperature at the location where each species is dominant and (2) to evaluate the association between those plant trait differences along the gradients and the photosynthetic pathway of the species. We based our analyses on the following hypothesis: Plant traits change predictably along climatic gradients representing selective forces of adaptation within growth forms. In a common environment, we cultivated C_3 and C_4 grasses from grasslands under different climatic conditions, and we measured a set of plant traits related to size and resource capture and utilization.

Methods

Site and species

The selected species are dominant in different areas of temperate grasslands of North and South America and include C_3 and C_4 photosynthetic pathways. Ten were from the Great Plains of North America and eight from South America (Table A1, App. 1). Individuals from North America were grown from seeds while those from South America were grown from tillers.

We cultivated 18 grass species in pots of 3 l filled with sand during eight months, from March to October 1997. Plants were grown in a garden at the Facultad de Agronomía, Universidad de Buenos Aires (FAUBA), Argentina (34°35.4' S; 58°28.8' W). At this site, climate significantly differs from the areas where grass species naturally grew: mean annual precipitation and temperature differences between FAUBA and the dry-coldest and wet-hottest areas were +775 mm, +8.2 ºC and –200 mm, –1ºC, respectively. After the acclimation period (for convenience and watering control) plants were moved to a semi-controlled greenhouse for the following two months. The environmental conditions of the greenhouse (temperature, relative humidity, and photoperiod regime) varied between a minimum of 18 °C, 55% and 13 h, and a maximum of 31 °C, 100% and 14 h. Since the greenhouse was a structure covered entirely by glass with a minimum transparency of 90%, the natural mean photosynthetic active radiation was $18,7 \pm 8,5$ $mol.m^{-2}.day^{-1}$ (we did not use artificial light). Plants were periodically rotated and watered, and weekly received 100 ml per pot of Hoagland solution. After two months of greenhouse growing, plants were harvested and trait measured. At the time of measurements, the experimental plants were all in a vegetative stage.

Climatic gradient

The environment from which the species were selected differed mostly in terms of mean annual precipitation and mean annual temperature. Although species occupied a range of climatic conditions, the value of precipitation and temperature assigned correspond to those conditions where each species had its maximal values of dominance (Table A1, App. 1). For species from the Great Plains of North America, we assigned the mean annual precipitation and mean annual temperature of the area where each species had its maximal relative productivity as an indicator of its dominance (Epstein et al. 1998). For species from Patagonia (South America), we assigned the precipitation and temperature where each species had the highest relative cover (Golluscio et al. 1982; León & Facelli 1981). Finally, we chose two

dominant species of the main community of the Flooding Pampa (South America, Perelman et al. 2001) and we assigned the precipitation and temperature values according to data of the closest meteorological station (Dolores, Prov. de Bs. As., Argentina, Anon. 1992). As expected, a significant and positive correlation between precipitation and temperature was found ($r = 0.67$, $n = 18$, $p = 0.002$).

Plant traits

We measured ten functional traits in all the species selected. The number of replicates (individuals) varied with species and among traits according to logistics and costs: from 5 to 8 for leaf length and width, blade and sheath areas, specific leaf area, and leaf dry matter content; 6 to 13 for vegetative height; 3 to 6 for tiller production rate; and 3 to 4 for nitrogen and carbon determinations (Table A1, App. 1). Vegetative height was measured to the highest point of the individual. Relative leaf elongation rate was calculated as the difference in the ln-transformed length of the last fully expanded leaf blade during a period of three days. Aerial plant biomass was harvested and separated into three parts: leaf blades, leaf sheaths, and stems. Leaf blade length, leaf blade maximum width (they will be termed 'leaf length' and 'leaf width' hereafter, respectively), leaf blade area, and leaf sheath area per tiller were measured (leaf area meter, Model Li-3000, LI-COR, Lincoln, NE, US). Specific leaf area was calculated as the ratio between leaf area blade and dry leaf mass blade. Biomass was stored in humid conditions in plastic boxes and fresh weight was determined no later than two hours from harvest to minimize the possible different loss of water among species (Garnier et al. 2001). Dry weight was determined on oven-dried plant material (75 °C for 48 h) and leaf dry matter content was calculated. Leaf $\delta^{13}C$ ratio was used as surrogate of water use efficiency index (Farquhar & Richards 1984). Isotope compositions were determined in the youngest fully expanded totally green leaf, on a SIRA Series II isotope ratio mass spectrometer (VG Isotech, Middlewich, UK) operated in direct inlet continuous flow mode after combustion of the samples in an elemental analyzer (Model NA1500, Series 1, Carlo Erba Instrumentazione, Milan, IT). The reference CO_2, calibrated against standard Pee Dee belemnite (PDB), was obtained from Oztech (Dallas, TX, US). A system check of analysis was achieved with interspersed working standards of cellulose, atropine and urea (Sigma, St. Louis, MO, USA). The accuracy of the measurement was $\pm 0.1‰$.

Additional traits were measured on subsets of species that covered the whole climatic gradient. For 15 species, we calculated the relative tiller production rate as the difference in the ln-transformed number of tillers per plant during a period of 24 days. For 12 species, we determined the nitrogen content in dry samples of fully expanded, totally green, and recently senesced leaves by Kjeldahl acid-digestion method, and expressed as concentrations (nitrogen per unit leaf mass) ignoring possible losses in leaf dry mass during senescence (Aerts 1996). Nitrogen resorption efficiency (NRE) (Killingbeck 1986) was calculated as follows:

$$\text{NRE (\%)} = \frac{(N_g - N_s) \times 100}{N_g} \qquad (1)$$

where N_g and N_s are the nitrogen concentration in green and senesced leaves, respectively (Table A1, App. 1).

Data analysis

We performed two complementary analyses. First, we described the general structure of C_3 and C_4 interspecific grass trait differences using Principal Component Analysis (PCA). A matrix of 18 species (all of them listed in Table A1, App. 1) by 9 plant traits was analyzed. We included traits that measured different plant properties (e.g. organs or attributes not trivially correlated): leaf length, leaf width, leaf blade area per tiller, leaf sheath area per tiller, plant height, specific leaf area, relative leaf elongation rate, leaf dry matter content and leaf $\delta^{13}C$ ratio. Since traits were measured in different units, data were standardized. Then, from the PCA output, we described the interspecific grass trait differences along the climatic gradient (objective 'a'). We fitted multiple regression models for the scores of species in PCA axes and climatic descriptors of the area where each species is dominant. Additionally, the relative importance of each trait in the PCA axes was revealed by the eigenvectors. We also evaluated the association between grass trait differences along the climatic gradient and the photosynthetic pathway of the species (objective 'b') including photosynthetic pathway as an additional explanatory variable of the variance not explained by climatic descriptors. Additionally, we performed t-tests for the scores of species in PCA axes. For biological interpretation we only considered those axes that were nontrivial; i.e. explained more variation than expected by chance (Jackson 1993).

Our second approach for data analysis was to quantify the relationships between each plant trait with climatic descriptors of the area where each species is dominant (objective 'a'), and with the photosynthetic pathway (objective 'b'). Firstly, we performed stepwise multiple regression analysis of each grass trait with respect to precipitation and temperature. Then, we included

photosynthetic pathway as an additional explanatory variable of the variance not explained by climatic descriptors. We tested whether the environmental effects were significant within the photosynthetic groups or in pooled data. Additionally, we compared each trait of C_3 and C_4 groups by t-tests. All references to statistical significance or significant differences are at $P \leq 0.05$.

Since we measured plant traits of grass species from different continents, our results could be confounded by intercontinental differences rather than differences related with climate and/or photosynthetic pathway. In the context of our paper, at least two sources of intercontinental differences are relevant. The first source is referred to different evolutionary history of grazing: The extinction of megaherbivores at the end of Pleistocene (about 10 000 yr ago) was greater in South America than in many parts of North America (Markgraf 1985). Consequently, grasslands have been subjected to different grazing intensities selecting different coordinated plant traits in each continent (e.g. Adler et al. 2004, see however Lauenroth 1998). The second source is related to different plant cultivation methods: North American species were grown from seeds while South American species were grown from planting tillers (see above). However, if this had been a relevant influence, we should have found differences between continents within a given photosynthetic pathway. We performed t-tests between each grass trait measured in C_3 species from North (n = 3) and from South American grasslands (n = 7) (similar tests were not possible for C_4 species because there is only one species from South America, see Table A.1, App. 1). Since we did not find any difference, we are confident that intercontinental differences were not a relevant source of plant trait differences in our analysis.
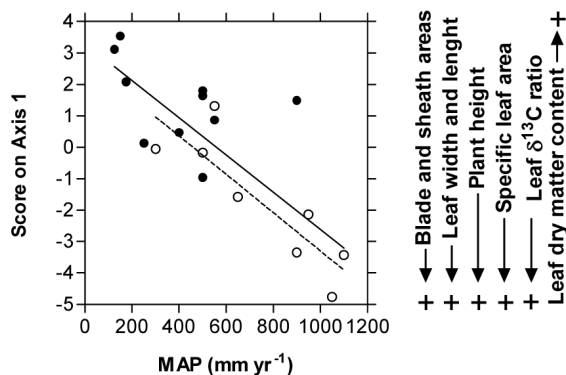
Results

The first axis of the Principal Component Analysis accounted for 56% of the total variation (31% expected by chance). Scores of species in Axis 1 were associated with mean annual precipitation when C_3 and C_4 grasses were considered together or C_4 species were considered alone (Fig. 1, Table 1). Axis 1 represented a change from C_3 grasses that have low leaf area, height, and specific leaf area, narrow and short leaves, low leaf $\delta^{13}C$ ratio, and high leaf dry matter content, to C_4 grasses with the opposite traits (Fig. 1, Table 2). A proportion of the variation along the gradient was related to the association between photosynthetic pathway and precipitation because C_3 species were concentrated towards the positive end and C_4 species towards the negative end (Fig. 1). For this reason, photosynthetic pathway was not a significant additional explanatory variable of the variance not explained by precipitation. Axis 2 explained less variation than expected from an uncorrelated data matrix (14% calculated vs. 20% expected by chance) therefore we did not interpret it biological (Jackson 1993).

All but two plant traits significantly varied with either mean annual precipitation or mean annual temperature (Fig. 2, Table 1). None of them significantly varied with photosynthetic pathway when it was included as an additional explanatory variable. Leaf length and width, plant height, and leaf dry matter content varied, respectively, almost 4, 40, 5, and 3-fold along the climatic gradient. Only when C_3 and C_4 grasses were considered together, leaf length and width, and plant height significantly increased with mean annual precipitation, while leaf dry matter content decreased along the precipitation gradient (Fig. 2a-d, Table 1). Leaf length and width and plant height were higher for C_4 than for C_3 grasses. In contrast, leaf dry matter content did not differ between C_3 and C_4 grasses (Fig. 2a-d, Table A1, App. 1).

Leaf area per tiller and specific leaf area varied almost 14 and 3-fold respectively along the climatic gradient. When C_3 and C_4 grasses were considered together or only for C_4 grasses, both traits significantly increase with mean annual precipitation. Both leaf area per tiller and specific leaf area were higher for C_4 than for C_3 grasses (Fig. 2e-f, Table 1 and Table A1, App. 1). Since the two components of leaf area per tiller (blade and sheath areas) were highly correlated ($r = 0.90$, $n = 18$, $p < 0.0001$), they behaved along the climatic gradients in a similar way as the total area (Fig. 2e and Table 1).

Leaf $\delta^{13}C$ ratio was positively related to precipitation only for C_4 grasses (Fig. 2g and Table 1). As expected, leaf $\delta^{13}C$ ratio was clearly different between C_3 and C_4 grasses (–30.2 and –13.1‰, respectively; Fig. 2g and Table A1, App. 1).

Nitrogen resorption efficiency varied almost 2-fold

Fig. 1. Scores of C_3 (●) and C_4 grass species (○) on Axis 1 of the Principal Component Analysis plotted against the mean annual precipitation of the area where each species is dominant (MAP; mm.yr^{-1}). Lines correspond to the significant regression models for all species together (continued line, $R^2 = 0.63$) or only for C_4 grasses (dashed line, $R^2 = 0.75$) (see Table 1). Labels display traits with the highest eigenvector weights on PCA axis 1 (Table 2).

Table 1. Relationships between the score of species on PCA axis 1 and 14 plant traits, and climatic variables. Models were performed with stepwise multiple regression analysis of each dependent variable with respect to both mean annual precipitation (MAP) and mean annual temperature (MAT) considering all species together or within each photosynthetic group. Note that only one independent variable (MAP or MAT) was significant in each model. Dash indicates models that were not significant.

Dependent variable	Species	n	Independent variables	R^2	P	Intercept	Regression coefficient
Score on PCA axis 1	All	18	MAP	0.63	<0.001	3.3	−0.006
	C_3	10	-				
	C_4	8	MAP	0.75	0.006	2.8	−0.006
Leaf length	All	18	MAP	0.30	0.02	13.9	0.014
	C_3	10	-				
	C_4	8	-				
Leaf width	All	18	MAP	0.49	0.001	0.1	0.0005
	C_3	10	-				
	C_4	8	-				
Plant height	All	18	MAP	0.32	0.01	16.2	0.03
	C_3	10	-				
	C_4	8	-				
Leaf dry matter content	All	18	MAP	0.31	0.02	45.3	−0.02
	C_3	10	-				
	C_4	8	-				
Leaf total area per tiller	All	18	MAP	0.55	0.001	−2.7	0.05
	C_3	10	-				
	C_4	8	MAP	0.85	<0.001	−16.1	0.07
Blade area per tiller	All	18	MAP	0.56	0.0003	−3.0	0.04
	C_3	10	-				
	C_4	8	MAP	0.82	0.002	−13.6	0.06
Sheath area per tiller	All	18	MAP	0.41	0.004	0.4	0.008
	C_3	10	-				
	C_4	8	MAP	0.72	0.008	−2.5	0.01
Specific leaf area	All	18	MAP	0.39	0.005	12.0	0.01
	C_3	10	-				
	C_4	8	MAP	0.65	0.02	11.9	0.01
Leaf $\delta^{13}C$ ratio	All	18	-				
	C_3	10	-				
	C_4	8	MAP	0.50	0.047	−14.9	0.002
N resorption efficiency	All	12	MAP	0.47	0.014	54.2	0.03
	C_3	7	-				
	C_4	5	-				
N concentration in green leaves	All	12	-				
	C_3	7	MAT	0.60	0.041	4.7	−0.2
	C_4	5	-				
N concentration in senesced leaves	All	12	MAT	0.48	0.012	1.5	−0.05
	C_3	7	-				
	C_4	5	-				
Relative leaf elongation rate	All	18	-				
	C_3	10	-				
	C_4	8	-				
Relative tiller production rate	All	15	-				
	C_3	9	-				
	C_4	6	-				

along the gradient. Once C_3 and C_4 grasses are pooled, a positive relationship between nitrogen resorption efficiency and precipitation was observed (Fig. 2h and Table 1). Nitrogen resorption efficiency was higher for C_4 than for C_3 grasses (75.2 and 63.0 %, respectively; Fig. 2h). Nitrogen concentration in green leaves, one of the components of the nitrogen resorption efficiency (Eq. 1), was negatively related to mean annual temperature only in the case of C_3 grasses (Fig. 2i and Table 1). We did not find differences in nitrogen concentration in green leaves between C_3 and C_4 grasses. The other component of nitrogen resorption efficiency, nitrogen concentration in senesced leaves, varied almost 4-fold along the gradient.
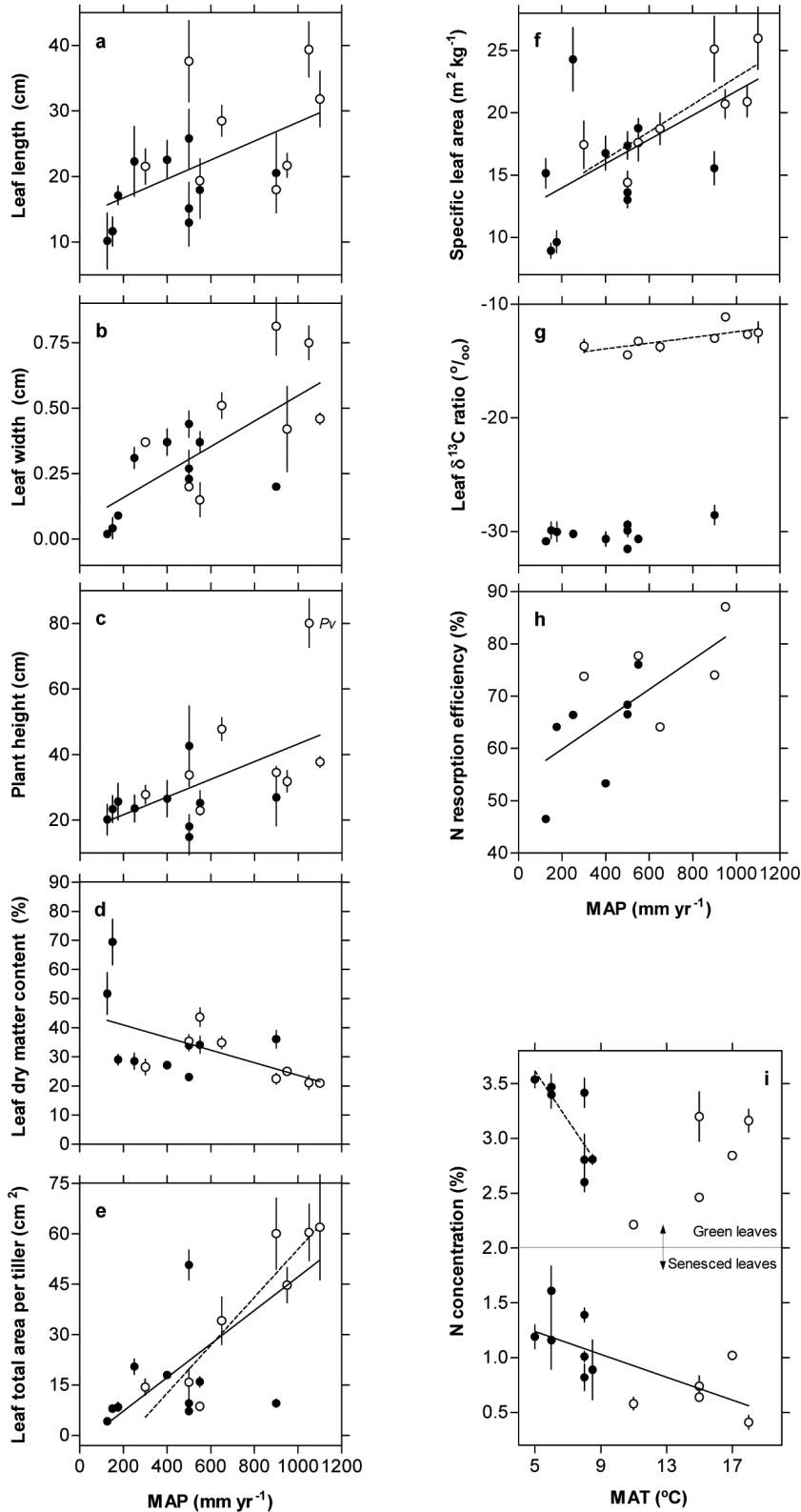
Fig. 2. Plant traits of C_3 (●) and C_4 grass species (○) plotted against the mean annual precipitation (MAP; mm.yr^{-1}) or mean annual temperature (MAT; ºC) of the area where each species is dominant. Lines correspond to the significant regression models for all species together (continued line in panels **a**, **b**, **c**, **d**, **e**, **f**, **h**, and **i**), only for C_4 grasses (dashed line in panels **e**, **f**, and **g**) or only for C_3 grasses (dashed line in panel **i**) (see Methods and Table 1). **a.** Leaf length ($R^2 = 0.30$); **b.** leaf width ($R^2 = 0.49$); **c.** plant height ($R^2 = 0.32$, although excluding *Panicum virgatum* (Pv) the regression model was significant ($R^2 = 0.21$, $n = 17$, $p = 0.06$); **d.** leaf dry matter content ($R^2 = 0.31$); **e.** leaf total area per tiller as the sum of blade and sheath areas per tiller ($R^2 = 0.55$ for all species and $R^2 = 0.85$ only for C_4 grasses); **f.** specific leaf area ($R^2 = 0.39$ for all species and $R^2 = 0.65$ only for C_4 grasses); **g.** leaf $\delta^{13}C$ ratio (for C_4 grasses $R^2 = 0.50$); **i.** nitrogen resorption efficiency ($R^2 = 0.47$); **i.** nitrogen concentration in green leaves (above, for C_3 grasses $R^2 = 0.60$) and in senesced leaves (below, $R^2 = 0.48$). Bars are standard error (except in panel **h** because the variable is synthetic), some of them hided by symbols. Some points were slightly displaced across the y-axis in order to avoid overlapping between them. However, the regression models were performed with unmodified data (Table 1 and Table A1 App. 1).

Table 2. Eigenvector weights of plant traits in Axis 1 of the Principal Component Analysis (56% variance accounted), obtained from a matrix of 9 traits × 18 grass species. Values are ranked in order of absolute magnitude along the axis.

Trait	Axis 1
Blade area	–0.41
Leaf width	–0.40
Sheath area	–0.37
Height	–0.35
Leaf length	–0.33
Specific leaf area	–0.32
Leaf δ^{13}C ratio	–0.31
Leaf dry matter content	0.30
Leaf elongation rate	0.05

When C_3 and C_4 grasses were considered together, nitrogen concentration in senesced leaves significantly decreased with mean annual temperature (Fig. 2i and Table 1). C_3 had more nitrogen concentration in senesced leaves than C_4 grasses (1.15 and 0.68% respectively; Table A1, App. 1). Therefore, a high proportion of nitrogen resorption efficiency variability along the climatic gradient was accounted for nitrogen concentration in senesced leaves, whereas the other component, nitrogen concentration in green leaves, did not explain a significant fraction of the variability in nitrogen resorption efficiency (Fig. 2h-i).

Two functional traits showed no significant differences between photosynthetic pathways or along the climatic gradient: Relative leaf elongation rate and relative tiller production rate (Table 1 and Table A1, App. 1).

Discussion

Plant traits measured under common conditions changed along the climatic gradient defined mainly by variation in precipitation in the areas where the species studied are dominant. Additionally, we found that C_3 species had different trait values than C_4 species (Fig. 1). Because C_3 and C_4 grasses showed a differential distribution along the precipitation and temperature gradients (Paruelo & Lauenroth 1996), a large proportion of trait variations along the gradient was related to the photosynthetic pathway. In this sense, ten traits significantly varied along the climatic gradient when all species were taken into account, five significantly varied when considering only C_4 species and none was significant within C_3 species (Table 1). This result shows that some features of the trait variation along the climatic gradient are significant by themselves within the C_4 group, even when the association between photosynthetic pathway and climate is removed from the data set. Specifically, leaf size, plant height, leaf area (absolute and specific), leaf δ^{13}C ratio, and a nitrogen conservation index (nitrogen resorption efficiency) increased while leaf dry matter content and nitrogen concentration in senesced leaves decreased as mean annual precipitation increased and, in general, were higher for C_4 than for C_3 grass species (Figs. 1 and 2). Based on these results, we did not reject the hypothesis that plant traits change predictably along environmental gradients representing selective forces of adaptation within growth forms.

Our results indicate a pattern of ecological specialization of dominant grasses along a climatic gradient. Dominant grasses in the dry-cold extreme of the gradient have a set of plant traits characteristic of low competitive environments: shorter plants with small leaf size and leaf area, low nitrogen reutilization, and high density tissues (Figs. 1 and 2). On the other hand, dominant grasses in wet-warm sites had opposite traits that characterized highly competitive environments. This finding confirms previous studies which show patterns of plant specialization that allow rapid acquisition of resources and are economically competitive (Díaz et al. 2004; Wright et al. 2004). However, these analyses were mainly based on *in situ* observations, which combined site effect with interspecific plant differences. Our work showed that such patterns of plant specialization remained when species are grown in a common environment.

Since C_3 and C_4 grasses have clear differences in carbon assimilation (Fig. 2g), leaf δ^{13}C ratio would have a higher relative weight in determining axis 1 scores than the other traits and, in consequence, leaf δ^{13}C would reveal the photosynthetic group. Then, we tested the effect of excluding leaf δ^{13}C ratio from the PCA matrix. Interestingly, the PCA results were very similar to those generated including leaf δ^{13}C ratio (respectively, 58 vs. 56% of variance explained by axis 1; 15 vs. 14% explained by the axis 2; and similar scoring of species and traits on axis 1 as Fig. 1 and Table 2 showed). These results emphasize the evidence that both groups of species have a syndrome related to size, resource capture and utilization. Similar syndromes were shown in a recent global study despite major differences in taxon, flora, climate, biogeography and land use (Díaz et al. 2004).

Interspecific plant trait differences were explained by mean annual precipitation or mean annual temperature (30 to 85%), and a proportion of those interspecific differences along the gradient was related to the association between photosynthetic pathway of the species and precipitation. These results should be compared with those based on the analysis of a global data set that showed a modest modulation of six key leaf traits by climate (Wright et al. 2004 analyzed leaf mass per area, photosynthesis rate, dark respiration rate, leaf nitrogen and phosphorus, and leaf lifespan in 2548 species from

219 families at 175 sites). For example, leaf mass per area (a measure of leaf dry-mass investment per unit of leaf area deployed, the inverse of specific leaf area) showed a weak relationship with mean annual precipitation and mean annual temperature ($R^2 = 0.002$; $R^2 = 0.10$, respectively). Considering only graminoid species (both C_3 and C_4 grasses, and sedges; 107 species from 24 sites), our re-analysis of Wright's data (2004) showed similar results; temperature and precipitation did not explain a higher proportion of the variance of those six key leaf traits: Leaf mass per area of graminoids showed a similar weak relationship with precipitation and temperature ($R^2 = 0.034$; $R^2 = 0.027$, respectively; in contrast with our results for specific leaf area variation along a precipitation gradient with $R^2 = 0.39$, see Table 1). Two reasons might explain the apparent differences between our findings and those of Wright et al. (2004) for graminoid species. First, most of the data compiled by Wright et al. (2004) corresponded to *in situ* measurements; consequently it is not possible to discriminate between genetic and phenotypic effects on trait values. Second, because some species occurred at more than one site, Wright's data set analyzed showed not only interspecific, but also intraspecific differences. That is why, for example, when we compared the same species (*A. gerardi* and *S. scoparium*) growing in different sites (Wright's data set) vs. in the garden (our data set), we found a higher coefficient variation in specific leaf area in Wright's data than in ours (35 vs. 18%, respectively).

Of course we can not discard biases associated to differences between both data sets. In the first place, the environmental gradient covered by Wright's data set was wider than the one used in our study, including graminoid species (grasses and sedges) from wetter or colder biomes than grasslands (20% and 25% of the species were from areas not included in our experiment, more than 1100 mm.yr^{-1} or less than 5 ºC, respectively). In the second place, Wright's data set included, compared to our experiment, a higher proportion of C_3 species (77 vs. 55%, respectively) and a bigger sample size ($n = 107$ vs. $n = 18$, respectively).

We showed that precipitation rather than temperature is the main control of the interspecific grass trait differences along the gradient (Figs. 1 and 2, and Table 1). Because both climatic variables are correlated along the gradient ($r = 0.67$, $n = 18$, $p = 0.002$) and the multiple stepwise models allowed discriminate the effects of each one separately, we assessed the importance of precipitation and/or temperature as controls of plant trait variation. In the same way, when the effects were isolated, precipitation rather than temperature was the principal control of structural and functional heterogeneity of grasslands, including abundance of life forms, primary productivity and nitrogen mineralization (Burke et al.

1997; Epstein et al. 1996, 1997; Jobbágy et al. 2002; Paruelo & Lauenroth 1996).

The C_3 and C_4 species have some different traits. Leaf size (length and width), plant height, leaf area (absolute and specific), and nitrogen resorption efficiency were lower for C_3 than for C_4 grasses, whereas leaf elongation, tiller production, nitrogen concentration in green leaves, and leaf dry matter content were not different between photosynthesis pathways. Although our data set does not encompass all the potential variations within the grassland biome (it is restricted to temperate areas), the results are partially different with previous studies of more extended lists based on root and leaf traits, including dominant and non-dominant grass species: C_3 grasses had denser leaves, thinner roots, and higher leaf and root nitrogen concentration than C_4 grasses (Craine et al. 2001, 2005; Wright et al. 2004). Because C_3 grasses selected in our study are mainly dominant in cold-dry sites and C_4 grasses in wet-warm grassland areas (Paruelo & Lauenroth 1996), we cannot conclude that the differences between C_3 and C_4 grasses disappeared due to a common environment where plants were grown, or if climate indirectly selected for different suite of traits.

Nitrogen resorption efficiency was associated with only one of its components: the nitrogen concentration in senesced leaves. Although nitrogen resorption efficiency was associated to precipitation whereas nitrogen concentration was related to temperature (Fig. 2h-i), the positive correlation between precipitation and temperature suggest a general trend between nitrogen resorption and its two components. We did not find a relationship between nitrogen resorption efficiency and nitrogen concentration in green leaves (Fig. 2h-i). Aerts (1996) showed that nitrogen resorption efficiency of graminoids was positive but weakly related to the nitrogen content of green leaves. Our results suggest that interspecific differences in nitrogen resorption efficiency are not mainly due to differences in nitrogen concentration in green leaves, but they are due to differences in the nitrogen concentration in leaves after senescence (Fig. 2 h-i) (resorption proficiency *sensu* Killingbeck 1986). While a similar pattern has been described for leaves of the Alaskan birch (*Betula papyrifera*) (Chapin & Moilanen 1991), to our knowledge, this is the first evidence for grass species. Thus, future research should focus on the biochemical and nutritional basis of nitrogen resorption controls for grasses, as previous studies for woody vegetation did (e.g. Chapin & Moilanen 1991).

Traits linked to competitive abilities suggest two indirect evidences of the well known fact that resource limitation increases as precipitation increased. First, leaf length, leaf width, specific leaf area, leaf area per tiller and plant height increased respectively 4, 40, 3, 14, and 5-fold while leaf dry matter content decreased 3-fold

along the precipitation gradient (Fig. 2). These results showed, in general, allometric relationships rather than scaling in direct proportion with one another ('isometric') (Wright et al. 2004) and support the hypothesis of a reduction in light availability at the soil surface and a concomitant increase in light competition as precipitation and productivity increase. At the community level, Lane et al. (2000) showed that both leaf area index and proportion of tall-stature grass species increased along a similar precipitation gradient (from 300 to 800 $mm.yr^{-1}$), suggesting that plants become leafier and taller along the gradient, as a previous paper showed for African graminoids, theory predicts and our results indicate (Coughenour et al. 1985; Falster & Westoby 2003, Fig. 2). Second, nitrogen resorption efficiency increased 2-fold and nitrogen concentration in senesced leaves decreased 4-fold as precipitation increased (Fig. 2). These results suggest a more conservative nitrogen use as precipitation increased and support the idea of an increase in nitrogen limitation toward the wettest extreme of the gradient as previous papers hypothesized (Aerts & Chapin 2000; Burke et al. 1997; Paruelo et al. 1999; Verón et al. 2002). As previous studies have shown (Aerts 1996), our results suggest that a strategy to maximize the nitrogen use of dominant grasses at the wettest extreme is to increase the residence time of nitrogen, i.e. the average time the nitrogen remains in the plant, since nutrients resorbed during senescence are retained in plants and used to support further growth.

Finally, our descriptions and analyses of structural and functional plant traits of grasses highlight some important issues. First, they provide a quantitative description to develop mechanistic hypotheses of processes operating at ecosystem level (e.g. limitation by light and nitrogen). Second, the data generated could be used to improve the calibration of simulation models of ecosystem functioning. Third, descriptions showed that climate by itself not only determines individual plant trait changes within a growth form (e.g. C_3 and C_4), generally higher in C_4 plants, but also affects replacement of growth forms with different traits.

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For App. 1, see below (online version)
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App. 1. Plant traits of 18 grass species in alphabetical order. Type: photosynthetic pathway; Area: area where each species is dominant or shows the highest relative abundance (Patagonia and Flooding Pampa are areas localized in the south portion of South America, remaining areas are in North America); MAP: mean annual precipitation of the area; MAT: mean annual temperature of the area; Leaf length: leaf blade length; Leaf width: leaf blade maximum width; Blade area: leaf blade area per tiller; Sheath area: leaf sheath area per tiller; Height: plant height; SLA: specific leaf area; Leaf elong.: relative leaf elongation rate; Tiller prod.: relative tiller production rate; LDMC: leaf dry matter content; Ng: nitrogen concentration in green leaves; Ns: nitrogen concentration in senesced leaves; $\delta 13C$: leaf $\delta 13C$ ratio. Mean values ± 1SD and number of replicates between brackets.

Species	Type	Area	MAP (mm year^{-1})	MAT (ºC)	Leaf length (cm)	Leaf width (cm)	Blade area (cm^2)	Sheath area (cm^2)
Agropyrum smithii Rydb.	C_3	Northern mixed prairie	500	8.5	25.80±4.46(7)	0.44±0.05(7)	40.95±7.13(7)	9.77±7.05(7)
Andropogon gerardii Vitman	C_4	Tallgrass prairie	1100	13.5	31.84±9.49(5)	0.46±0.05(5)	51.82±28.78(5)	10.16±6.22(5)
Bouteloa curtipendula (Michx.) Torr.	C_4	Southern mixed prairie	650	17	28.52±5.74(6)	0.52±0.12(6)	30.03±16.03(6)	4.14±1.56(6)
Bouteloa gracilis (H.B.K.) Lag. Ex Griffiths	C_4	Shortgrass steppe	500	16	37.62±15.17(6)	0.20±0.001(6)	11.79±7.85(6)	4.07±2.36(6)
Bromus setifolius Presl	C_3	Patagonia	400	6	22.57±2.96(7)	0.37±0.05(7)	14.14±2.08(7)	3.91±1.01(7)
Buchloe dactyloides (Nutt.) Engelm.	C_4	Shortgrass steppe	550	15	19.38±8.10(6)	0.16±0.04(6)	7.13±2.89(6)	1.52±0.41(6)
Danthonia montevidensis Hack. et. Arech.	C_3	Flooding Pampa	900	15	20.55±6.05(6)	0.20±0.001(6)	8.23±3.14(6)	1.36±0.47(6)
Festuca pallescens (St. Yves) Parodi	C_3	Patagonia	500	6	12.98±3.57(7)	0.28±0.07(7)	7.83±4.44(7)	1.75±0.61(7)
Hilaria jamesii (Torr.) Benth.	C_4	Shortgrass steppe	300	11	21.56±7.07(7)	0.37±0.08(7)	11.16±5.72(7)	3.26±0.88(7)
Hordeum comosum Presl	C_3	Patagonia	250	6	22.33±5.32(6)	0.32±0.04(6)	16.01±4.85(6)	4.51±1.45(6)
Panicum virgatum L.	C_4	Tallgrass prairie	1050	13.5	39.40±11.13(7)	0.76±0.17(7)	50.79±18.89(7)	9.62±3.78(7)
Paspalum dilatatum Poir.	C_4	Flooding Pampa	900	15	18.01±2.36(8)	0.81±0.31(8)	48.13±24.25(8)	11.90±5.95(8)
Poa ligularis Nees ap. Steud.	C_3	Patagonia	175	8	17.16±1.43(7)	0.09±0.007(7)	5.60±2.55(7)	2.87±1.15(7)
Schizachyrium scoparium (Michx.) Nash-Gould	C_4	Tallgrass prairie	950	18	21.73±4.83(7)	0.43±0.05(7)	31.96±9.22(7)	12.79±5.81(7)
Stipa comata Trin. et. Rupr.	C_3	Northern mixed prairie	500	5	15.13±4.02(7)	0.22±0.04(7)	5.73±1.75(7)	1.52±0.39(7)
Stipa humilis Cav.	C_3	Patagonia	125	8	10.18±4.26(6)	0.02±0.001(6)	2.65±1.32(6)	1.62±0.94(6)
Stipa speciosa Trin. et. Rupr.	C_3	Patagonia	150	7	11.64±2.21(5)	0.04±0.004(5)	5.42±1.40(5)	2.57±0.72(5)
Stipa viridula Trin.	C_3	Northern mixed prairie	550	8	18.47±4.36(6)	0.37±0.04(6)	14.39±3.70(6)	1.57±0.35(6)

	Height (cm)	SLA (m^2 kg^{-1})	Leaf elong. (cm cm^{-1} day^{-1})	Tiller prod. (day^{-1})	LDMC (%)	N$_g$ (%)	N$_s$ (%)	δ^{13}C (‰)
A. smithii	42.71±12.10(7)	13.19±1.62(7)	0.212±0.12(7)	0.030±0.027(6)	34.98±3.96(7)	2.81±0.08(3)	0.89±0.47(3)	-31.55±0.31(3)
A. gerardi	37.80±5.20(10)	25.99±5.57(5)	0.155±0.16(6)	N/A	20.97±2.92(5)	N/A	N/A	-12.49±0.91(3)
B. curtipendula	47.85±9.35(7)	18.72±3.11(6)	0.175±0.17(7)	0.051±0.023(6)	34.89±5.10(6)	2.84±0.06(3)	1.02±0.07(3)	-13.73±0.44(3)
B. gracilis	33.80±10.84(10)	14.42±2.20(6)	0.260±0.18(7)	0.016±0.007(3)	35.37±5.54(6)	N/A	N/A	-14.45±0.16(3)
B. setifolius	26.57±5.48(8)	16.77±3.59(7)	0.255±0.12(8)	0.043±0.019(5)	27.16±3.38(7)	3.45±0.21(3)	1.61±0.39(3)	-30.68±0.48(3)
B. dactyloides	23.00±3.65(7)	17.60±3.60(6)	0.331±0.19(6)	0.036±0.014(3)	43.68±7.75(6)	3.20±0.39(3)	0.74±0.16(3)	-13.26±0.15(3)
D. montevidensis	27.00±8.69(7)	15.56±3.27(6)	0.248±0.29(6)	0.042±0.033(3)	36.05±7.38(6)	N/A	N/A	-28.56±1.46(3)
F. pallescens	18.10±3.60(10)	13.38±2.89(7)	0.148±0.25(6)	0.030±0.005(3)	23.07±2.31(7)	N/A	N/A	-29.94±0.89(3)
H. jamesii	27.83±6.85(6)	17.44±5.00(7)	0.256±0.26(6)	0.005±0.020(3)	26.55±7.15(7)	2.21±0.06(3)	0.58±0.10(3)	-13.69±0.59(4)
H. comosum	23.57±4.14(8)	24.30±6.24(6)	0.326±0.42(7)	0.018±0.040(5)	28.54±6.77(6)	3.45±0.20(3)	1.16±0.46(3)	-30.22±0.45(3)
P. virgatum	80.14±23.38(10)	20.90±3.26(7)	0.301±0.22(7)	N/A	21.12±6.33(7)	N/A	N/A	-12.66±0.30(3)
P. dilatatum	34.57±5.95(10)	25.13±7.39(8)	0.232±0.23(7)	0.016±0.014(3)	22.49±5.29(8)	2.46±0.03(3)	0.64±0.06(3)	-13.00±0.27(3)
P. ligularis	25.69±5.64(13)	9.63±2.31(7)	0.245±0.22(7)	0.023±0.014(3)	29.08±4.89(7)	2.81±0.40(3)	1.01±0.08(3)	-30.04±1.51(3)
S. scoparium	31.87±9.29(8)	20.72±3.00(7)	0.159±0.15(6)	0.043±0.031(5)	25.04±2.10(7)	3.16±0.18(3)	0.41±0.11(3)	-11.11±0.22(3)
S. comata	14.85±5.46(9)	17.37±2.91(7)	0.166±0.27(6)	0.020±0.055(3)	33.92±4.84(7)	3.54±0.13(3)	1.19±0.19(3)	-29.41±0.61(3)
S. humilis	20.18±4.64(11)	15.15±2.89(6)	0.322±0.26(7)	0.027±0.007(3)	51.74±17.38(6)	2.60±0.15(3)	1.39±0.11(3)	-30.87±0.17(3)
S. speciosa	23.42±4.11(7)	8.93±1.35(5)	0.206±0.43(5)	N/A	69.45±17.48(5)	N/A	N/A	-29.91±1.29(3)
S. viridula	25.28±3.80(11)	18.77±1.74(5)	0.162±0.18(8)	0.018±0.027(3)	34.19±7.04(6)	3.42±0.23(3)	0.82±0.21(3)	-30.68±1.10(3)