



Photosynthetic pathway variation among C_4 grasses along a precipitation gradient in Argentina

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ABSTRACT

Aim Based on the biochemical and physiological attributes of C_4 grasses, and on the close association between decarboxylation pathways and the taxa in which they evolved, the hypotheses tested were: (1) that C_4 grasses would become progressively more abundant as precipitation decreased, with grasses of the NADP-me subtype more abundant in wetter sites and those of the NAD-me subtype more common in arid regions; and (2) that the distribution of grass subfamilies would also be correlated with annual precipitation.

Location The study was conducted along a precipitation gradient in central Argentina, from the eastern Pampas (>1000 mm year⁻¹) to the western deserts and semi-deserts near the Andes (<100 mm year⁻¹).

Methods Percentage of species and relative cover of C_3 and C_4 grasses (including C_4 subtypes) in local floras from 15 lowland sites of central Argentina were obtained from our own unpublished data and from recently published floristic surveys. Pearson correlation coefficients were obtained between grass distribution parameters and the available climatic data.

Results The percentage of C_4 grasses increased towards the arid extreme and showed a strong negative correlation with annual rainfall ($r = -0.74$, $P < 0.01$). Within the C_4 subtypes, the NADP-me species showed a higher proportional representation at the wetter extreme, whereas the representation of NAD-me species increased towards the more arid extreme. The relationship of PEP-ck species with climatic parameters in central Argentina was less evident. The distributions of the Panicoideae and Chloridoideae subfamilies along the precipitation gradient were diametrically opposed, with the Panicoideae positively ($r = 0.86$, $P < 0.001$) and the Chloridoideae negatively ($r = -0.87$, $P < 0.001$) correlated with annual precipitation.

Main conclusions Our data are consistent with the broad observation that C_4 grasses tend to dominate in areas where the wet season falls in the warmer summer months. In agreement with previously reported results for Africa, Asia, Australia and North America, we describe here for the first time a significant relationship between annual precipitation and the prevalence of the NADP-me and NAD-me photosynthetic pathways along climatic gradients for the Neotropics. We also report for the first time that correlations between C_4 species and annual rainfall are stronger when the relative cover of grass species is considered. The association of grass subfamilies Panicoideae and Chloridoideae with rainfall is as strong as that recorded for the NADP-me and NAD-me variants, respectively, suggesting that characteristics other than decarboxylation type may be responsible for the geographic patterns described in this study.

Keywords

Central Argentina, Chloridoideae, C_4 grasses, C_4 photosynthesis, NADP-me photosynthesis, NAD-me photosynthesis, PEP-ck photosynthesis, Panicoideae.

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INTRODUCTION

The fundamental polarization of grass species into C_3 or C_4 photosynthetic types, with their distinct ecophysiologicals, is manifested in differential phytogeographical distributions (Cavagnaro, 1988; Hattersley, 1992; Ehleringer & Monson, 1993; Mooney & Ehleringer, 1997; Collatz *et al.*, 1998; Sage *et al.*, 1999b; Sage & Kubien, 2003). C_4 grasses are generally more abundant in warm environments (Sage & Sage, 2002), while C_3 grasses predominate in cool climates (Bianchin Belmonte & Rugolo de Agrasar, 2002). The corollary is that C_4 grasses usually outgrow C_3 grasses in the Tropics and Subtropics, and C_3 grasses outgrow C_4 grasses in temperate to cool-temperate areas (Keeley & Rundel, 2005). These distribution patterns are remarkably consistent at a large range of spatial scales, and in all the parts of the world where they have been studied (see reviews by Hattersley, 1992; Hattersley & Watson, 1992; Ehleringer & Monson, 1993; Ehleringer *et al.*, 1997; Sage *et al.*, 1999a). However, some studies indicate that the floristic cross-over point (equal percentages of C_3 and C_4 species) is not always coincident with the ground-cover cross-over point (equal cover of C_3 and C_4 species) (Rundel, 1980; Cabido *et al.*, 1997). Moreover, Epstein *et al.* (1997) have shown that the percentage of species having C_3 and C_4 photosynthetic pathways in the Great Plains (USA) is not correlated with the percentage of production accounted for by these types.

Three major decarboxylation subtypes have been identified within the C_4 photosynthetic pathway. Each C_4 subtype shows not only morphological but also biochemical differences (Sage, 1999). The three subtypes are named after the enzymes that decarboxylate the four-carbon acids transported into the bundle sheath: NADP-malic enzyme (NADP-me subtype), NAD-malic enzyme (NAD-me subtype), and PEP carboxylase (PEP-ck or PCK subtype) (Sage, 1999). Phytogeographical analysis of subtypes within the C_4 grasses reveals that the proportional representation of both aspartate (NAD-me or PEP-ck subtypes) and malate (NADP-me) formers is also correlated with climatic variables (Hattersley, 1992; Ehleringer & Monson, 1993; Ghannoum *et al.*, 2001). Ellis *et al.* (1980) for Namibia, and Vogel *et al.* (1978; 1986) for the Sinai, Negev and Judean deserts found that NADP-me-type species formed a greater proportion of the C_4 grass flora at the high-rainfall extremes of climatic gradients. In contrast, the proportional representation of NAD-me grasses was higher than any other C_4 type in the arid extremes of climatic gradients. The distribution of PEP-ck type grasses was not as clearly related to climatic parameters as those of the other two subtypes, and they tended to increase in number of species at the central part of the rainfall gradient. Hattersley (1992) reported that the proportional representation of both NADP-me and PEP-ck types in Australia was strongly and positively correlated with annual rainfall, whereas that of the NAD-me C_4 type showed a strong negative correlation with it. More recently, Taub (2000) found for the United States that the number of grass species that use the NADP-me variant of C_4 photosynthesis increased

with increasing annual rainfall, while that of grass species using the NAD-me and PEP-ck variants showed the opposite trend. Published analyses of the distribution of different types of C_4 grasses along climatic gradients are based almost entirely on presence/absence data (Prendergast, 1989). Very few studies have quantified the performance of photosynthetic C_4 variants in relation to climate in terms of cover or biomass, despite the importance of such information for understanding the adaptive characteristics of the different pathways (Boutton *et al.*, 1980; Tix & Charvat, 2005).

Even before the discovery of the C_4 photosynthesis pathways (Hatch & Slack, 1966), Hartley (1958) and Hartley & Slater (1960) noted some differences in the geographic distribution patterns of grass subfamilies, with the Eragrostoideae (= Chloridoideae) preferentially distributed in drier areas and the Andropogoneae (a tribe within the subfamily Panicoideae) in wetter sites. Taub (2000) has confirmed this pattern, reporting a strong association of grass subfamilies with annual precipitation. On the other hand, a strong association of C_4 variants has been reported among the various grass subfamilies (Hattersley, 1987; Sage *et al.*, 1999b). Most of the species within the Chloridoideae are of either the NAD-me or PEP-ck type, while in the subfamilies Arundinoideae and Panicoideae the NADP-me variant predominates. The results reported by Taub (2000) suggest that characteristics other than photosynthetic subtype, which differ among grass subfamilies, may be responsible for the differences in distribution of members of these subfamilies along environmental gradients.

In this study we examine the distribution of C_3 and C_4 grasses (the latter considered collectively and separated into the three photosynthetic subtypes) in 15 lowland sites of central Argentina in relation to climatic parameters. As mentioned above, the geographic distribution of C_4 variants has been reported for grass floras from North America, Africa and Australia, but detailed studies in the Neotropics are still lacking. In addition, we consider the relationships of climatic variables with the distribution of C_4 grass subfamilies. Based on the biochemical and physiological attributes of C_3 and C_4 grasses, and on the close association between the decarboxylation pathways and the taxa in which they evolved, we predicted that: (1) C_4 grasses should become progressively more abundant in terms of the proportion of species and cover as precipitation decreases along the gradient; (2) the distributions of the various C_4 photosynthetic subtypes should differ from each other along the rainfall gradient, with grasses of the NADP-me subtype more abundant in wetter sites and those of the NAD-me subtype more abundant in arid regions; and (3) the distribution of grass subfamilies should be correlated with annual precipitation.

METHODS

The study area in central Argentina falls within four phytogeographical provinces: Pampa, Espinal, Chaco and Monte (Cabrera, 1976). Vegetation types comprise climatic grasslands to the east, xerophytic woodlands in the central part of the

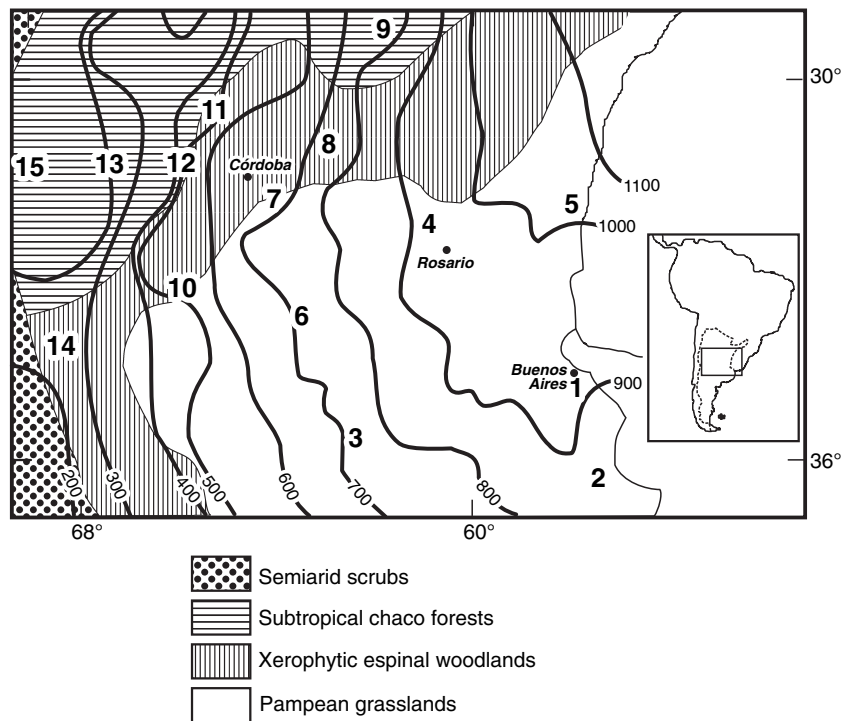


Figure 1 Location of the study area, and major vegetation types and annual isohyets (Morello, 1986) in central Argentina. The numbers from 1 to 15 on the map refer to the following study sites (sources for the distribution of C₃ and C₄ grasses are mentioned for each site). 1, Eastern Pampa, Buenos Aires Province: Eskuche (1992); 2, Flooded Pampa, Buenos Aires Province: Burkart *et al.* (1990), complemented with data from Eskuche (1992); 3, Partido de Pellegrini, Buenos Aires Province: Eskuche (1992); 4, Rosario Department, Santa Fe Province: authors' own unpublished data; 5, Parque Nacional El Palmar, Colón, Entre Ríos Province: Eskuche (1992); 6, Juárez Celman Department, Córdoba Province: authors' own unpublished data; 7, Río Segundo Department, Córdoba Province: authors' own unpublished data; 8, Tulumba Department, Córdoba Province: authors' own unpublished data; 9, Tostado, Santa Fe Province: Eskuche (1992); 10, Villa Mercedes, San Luis Province: Anderson *et al.* (1970), complemented and updated with authors' own unpublished data; 11, Cruz del Eje Department, Córdoba Province: authors' own unpublished data; 12, Pocho Department, Córdoba Province: authors' own unpublished data; 13, Rosario Vera Peñaloza Department, La Rioja Province: authors' own unpublished data; 14, Ñacuñán Provincial Reserve, Mendoza Province: Roig (1971), complemented and updated with authors' own unpublished data; 15, Ullúm Valley, San Juan Province: authors' own unpublished data.

gradient, and xerophytic open shrublands in the arid western extreme (Fig. 1). According to the Koeppen-Geiger climatic classification (Koeppen & Geiger, 1954), the climate in the study area is temperate/warm-temperate to subtropical, with a marked rainfall gradient from east (annual rainfall >1000 mm) to west (annual rainfall <100 mm). Temperature tends to increase towards the central and western parts of the range, but the trend is not as clear as that reported for annual rainfall. Except for sites 1 and 2 (Eastern and Flooded Pampa), the area as a whole falls in the summer rainfall (monsoonal) regime, with the result that the active growing season of the vegetation occurs during the warmer summer months (53–80% of the rain falls from November to March).

Distribution data for C₃ and C₄ grasses in local floras for the 15 sites studied in central Argentina were obtained from our own unpublished and other authors' published recent floristic surveys (see Fig. 1 for references). Only surveys carried out during the growing season, when all the species were present and likely to be recognized, were considered. All sites used for this study were included in the analysis only if they had at least

15 relevés (vegetation samples) comprising a minimum of 20 grass species. A minimum number of relevés was required in order to provide a complete floristic representation of the site. A minimum number of grass species was required so that the proportion of species at a site and within a single photosynthetic pathway would not be unduly influenced by the presence/absence of a single species. Another prerequisite for the acceptance of a site was that climatic data were available from weather stations located in the proximity of the site. Notwithstanding these prerequisites, the area surveyed around each site could still vary in size; for this reason, comparisons of species numbers (within pathway types and subtypes) between sites were performed on the basis of percentages of the C₃ and C₄ grasses in local floras, rather than on the absolute number of species.

The percentages of C₃ and C₄ grass species were calculated for each of the 15 sites. The C₄ photosynthetic pathway was identified by examination of the Kranz anatomy in cross-sections of fresh and herbarium specimens and from the literature (Smith & Epstein, 1971; Sánchez & Arriaga,

1990; Hattersley & Watson, 1992). The C₄ grasses were further classified as being either malate (NADP-me) or aspartate formers, and, if the latter, as either NAD-me or PEP-ck subtypes. The C₄ subtype was predicted from leaf structural observations based on the anatomy and cytology of the bundle sheath and mesophyll cells and from the literature (Hattersley & Watson, 1976; Brown, 1977; Zuloaga, 1987; Sage *et al.*, 1999a). Recent C₄ biochemical typing (Prendergast *et al.*, 1986, 1987) has shown that some of the predictions of biochemical variation based on leaf structure were erroneous (Hattersley, 1992). Thus, the C₄ typing based on leaf structural variations was retyped for taxa with biochemical typing available in the literature (Prendergast *et al.*, 1986, 1987; Prendergast & Hattersley, 1987; Hattersley, 1992; Sage *et al.*, 1999b; Giussani *et al.*, 2001). Species that could not be precisely identified as PEP-ck or NAD-me were considered for the analyses based on family membership and C₃ vs. C₄ percentages, but excluded from those that compared C₄ subtypes.

The percentages of C₃ and C₄ grass species were obtained for each site and expressed as percentages of the total local grass flora. Relative cover was also calculated for each grass species on the basis of the central class cover values obtained from cover-abundance values recorded in the field using the Braun-Blanquet combined scale (Braun-Blanquet, 1932). Cover values of single species (recorded in each relevé) were averaged to obtain a single value per site. The percentage of species and the relative cover of species representing the malate and aspartate photosynthetic subtypes were expressed as a percentage of the total C₄ grass species number and cover for each of the 15 sites.

Five climatic variables (annual rainfall, summer rainfall and three temperature variables) and two pluviothermic indices were used in this study (Table 1). These variables were selected because several studies have shown strong correlations between the distribution of photosynthetic pathways and precipitation and temperature (Teeri & Stowe, 1976; Vogel *et al.*, 1986; Cabido *et al.*, 1997), and also because they were the only available variables for all the weather stations included in this study. Climatic parameters were used to calculate Emberger's pluviothermic quotient (Emberger, 1930):

$$E = P \times 100 / (T_{mx} + T_{mn})(T_{mx} - T_{mn})$$

and Lang's rain factor (Lang, 1920):

$$L = P/T,$$

where *P* is the mean annual rainfall (mm), *T* is the mean annual temperature (°C), *T_{mx}* is the mean temperature of the warmest month (°C), and *T_{mn}* is the mean temperature of the coldest month (°C). Both indices express the relationship between temperature and rainfall and provide information about the effective precipitation. Ideally, these indices would relate annual rainfall to evaporation, but, because evaporation data are not readily available for our field sites, we used Lang's and Emberger's quotients, which derive moisture estimates from temperature instead of evaporation. Pearson's correlation coefficients were calculated for the relationships between distribution parameters (percentage of C₄ and C₃ species, relative cover of C₄ and C₃ species, percentage of species and relative cover of C₄ variants) and climatic variables. Correlation coefficients were also calculated between Emberger's and Lang's indices and the C₃ and C₄ distribution parameters.

Subfamily membership follows Watson & Dallwitz (2005). Complete grass species lists for the 15 sites are available upon request.

RESULTS

Distribution of C₃ and C₄ grasses

In 12 out of the 15 study sites the C₄ grasses showed a higher percentage of species than the C₃ type (Fig. 2a; Table 2). When relative grass cover was taken into account, C₄ grasses predominated in 10 out of the 15 study sites included (Fig. 2b; Table 2). The highest percentages of C₄ grasses occurred at the sites with the highest temperatures (sites 7 to 15) and /or lowest annual rainfall (sites 9 to 15). Higher percentages of C₃ grasses were found in the wetter extreme of the gradient in the Eastern and Flooding Pampa (sites 1 and 2 respectively; Table 2) than in the drier locations. The high C₃ abundance

Table 1 Climatic parameters for the 15 sites in central Argentina. For locations of the 15 sites see Fig. 1. Data for sites 1, 3 and 9 were taken from De Fina (1992); data for sites 4, 5, 7, 8, 9 and 15 from the National Meteorological Service (<http://www.meteofa.mil.ar/>); and data for sites 2, 6, 10, 11, 12, 13 and 14 from unpublished records of the Argentine Railways.

Site	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
AR	996	972	743	933	1060	879	870	787	771	567	574	503	322	279	92
SR	400	417	450	543	585	594	644	606	447	439	432	396	256	201	63
AAT	16.7	15.1	16.1	17.3	17.7	16.1	17.2	18.1	19.5	17.2	18.6	18.2	19.6	15.6	17.9
JAT	24.4	21.8	24.0	24.8	25.5	23.4	23.5	24.4	27.0	24.3	25.7	26.0	27.1	24.2	27.0
JUT	9.6	9.1	8.2	10.0	10.6	8.8	10.0	10.8	13.1	9.4	11.4	11.0	10.8	7.1	7.8
L	60	64	46	54	60	55	51	44	39	33	31	28	16	18	5
E	198	248	146	181	197	187	192	164	138	113	108	91	52	52	14

AR: annual rainfall (mm); SR: summer rainfall (mm); AAT: annual average temperature (°C); JAT: January mean temperature (°C); JUT: July mean temperature (°C); L: Lang's rain factor; E: Emberger's pluviothermic quotient.

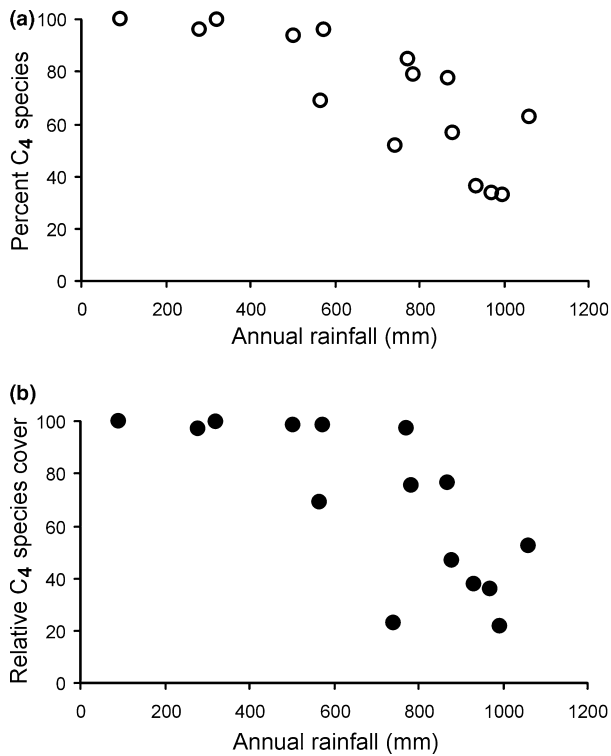


Figure 2 C₄ grass-species (a) composition (percentage of grass species) and (b) relative grass cover at 15 sites along a precipitation gradient in central Argentina.

coincided with the region where rainfall is abundant in winter and spring and only about 40% of the annual rainfall occurs during the summer months. In all the other sites, summer rainfall represents between 53% and 80% of the total annual

precipitation (Table 1). C₃ species were rare in sites with annual rainfall ≤ 600 mm and annual average temperature $> 17^{\circ}\text{C}$ (site 14, which has very low annual rainfall, is an exception). C₃ species were absent in sites 13 and 15 (Table 2), where annual rainfall is ≤ 300 mm and the average temperature of the warmest month exceeds 26°C .

The percentage of C₄ grass species in local floras was negatively related to annual rainfall (Table 3). Although to a lesser degree than rainfall, annual average temperature and the temperature of the warmest month also showed significant relationships with the percentage of C₄ species. C₄ relative grass cover showed a similar pattern to that of the percentage of C₄ species (Fig. 2), and the cross-over point with C₃ grass cover occurred at approximately 800 to 850 mm annual rainfall. The percentages of C₃ species in local floras were well correlated with all climatic parameters, except with summer rainfall and the average temperature of the coldest month (Table 3). The percentages of C₃ grasses were significantly negatively correlated with annual average temperature, and the relationship was even closer with the average temperature of the warmest month. This means that, in sites where the summer is warmer, the proportion of C₃ grasses decreases. However, a stronger correlation was found between the number of C₃ grasses and annual rainfall than between the number of C₃ grasses and temperature.

Distribution and subfamily membership of NADP-me, NAD-me and PEP-ck subtype species

NADP-me species dominated the local floras of C₄ grasses where rainfall was highest, progressively declining in importance with decreasing rainfall (Tables 2 and 3; Fig. 3). The NADP-me type was significantly and positively correlated with

Table 2 Distribution of C₄ grasses (percentage of the total number of grass species and percentage ground cover of grass species) for 15 sites in central Argentina. For the locations of the 15 sites see Fig. 1. Values for C₃ species are not shown as they are the complement of those for C₄ grasses.

Sites	C ₄		NADP-me		NAD-me		PEP-ck	
	Percentage of grass sp.	Percentage cover	Percentage of grass sp.	Percentage cover	Percentage of grass sp.	Percentage cover	Percentage of grass sp.	Percentage cover
1	33.3	21.6	45.5	92.4	45.5	6.8	9.0	0.7
2	34.0	35.5	50.0	80.1	38.9	19.8	11.1	0.1
3	51.9	23.3	64.3	63.5	28.6	25.6	7.1	9.8
4	36.3	37.8	50.0	62.6	37.5	20.9	12.5	16.4
5	62.5	53.1	84.0	98.4	8.0	1.4	8.0	0.2
6	57.2	46.9	56.3	41.8	25.0	53.9	18.7	4.3
7	78.0	76.8	68.7	71.1	28.1	22.1	3.2	6.8
8	79.4	76.4	37.0	26.0	40.7	60.4	22.3	13.6
9	84.8	97.9	64.4	94.7	17.8	2.5	17.8	2.7
10	68.6	69.1	54.2	60.9	41.7	35.2	4.1	3.8
11	96.4	99.8	37.0	36.1	44.4	53.0	18.6	10.9
12	93.7	99.2	40.0	39.7	46.7	54.5	13.3	5.6
13	100.0	100.0	36.4	19.7	50.0	79.3	13.6	1.0
14	95.5	98.2	28.6	29.9	66.7	69.9	4.7	0.1
15	100.0	100.0	30.0	15.8	60.0	83.7	10.0	0.5

Table 3 Pearson's correlation coefficient between grass distribution parameters (percentage of the total number of grass species and percentage cover of grass species) and climatic variables and pluviothermic indices for 15 sites in central Argentina. C₃ grass numbers are not included because they show exactly the complementary pattern to that of C₄ grasses. For definitions of climatic variables see Table 1.

Variable	Total grass species		C ₄ grass species					
	C ₄		Malate formers			Aspartate formers		
	Percentage sp.	Cover	%NADP-me cov	NADP-me	%NAD-me	NAD-me	%PEP-ck	PEP-ck
AR	-0.74 ***	-0.74**	0.70**	0.82***	-0.75**	-0.87***	0.08 NS	0.23 NS
SR	-0.36 NS	-0.43 NS	0.65**	0.55*	-0.75**	-0.66**	0.21 NS	0.51*
AAT	0.62*	0.63**	-0.07 NS	-0.07 NS	-0.09 NS	0.05 NS	0.44 NS	0.13 NS
JAT	0.73**	0.65*	-0.21 NS	-0.25 NS	0.12 NS	0.27 NS	0.25 NS	-0.09 NS
JUT	0.15 NS	0.29 NS	0.26 NS	0.42 NS	-0.47 NS	-0.46 NS	0.54*	0.22 NS
L	-0.82***	-0.80***	0.67**	0.78***	-0.69**	-0.82***	0.03 NS	0.18 NS
E	-0.84***	-0.75**	0.62**	0.76***	-0.66**	-0.79***	0.05 NS	0.18 NS

P* < 0.05; *P* < 0.01; ****P* < 0.001.

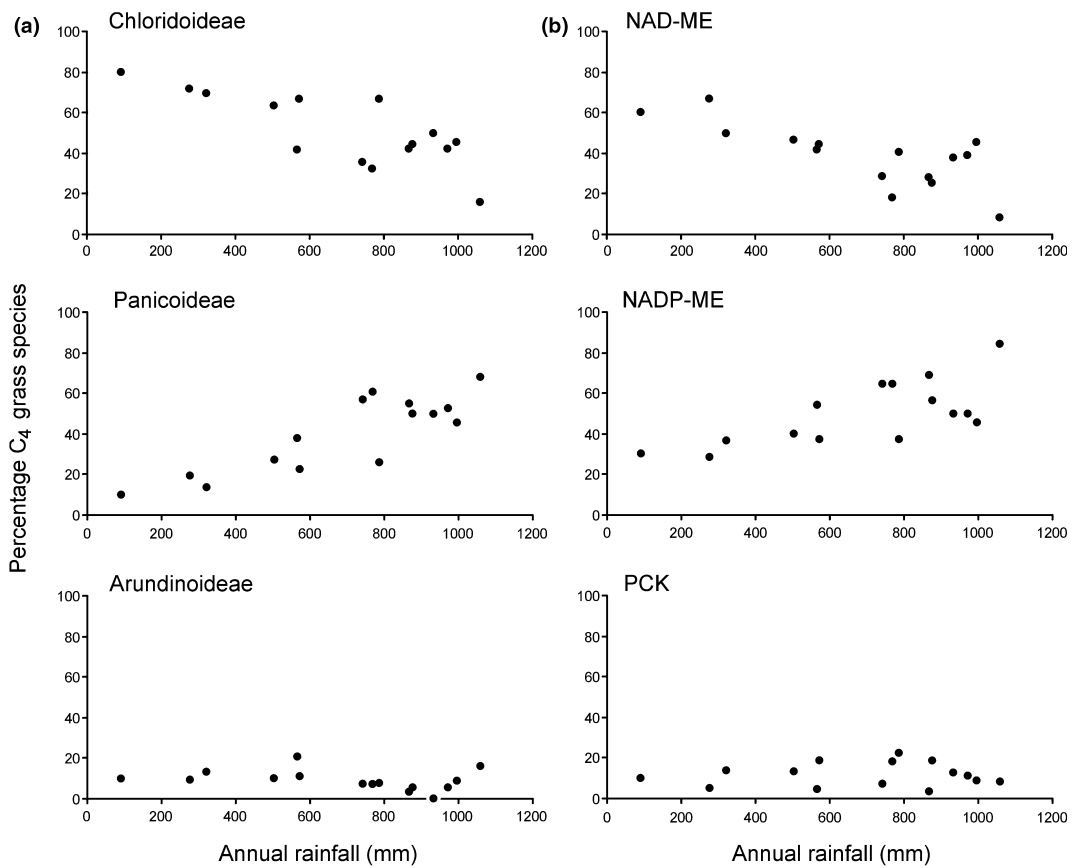


Figure 3 Relationships between (a) annual precipitation and the percentage of C₄ grasses in the Chloridoideae, Panicoideae and Arundinoideae subfamilies, and (b) annual precipitation and the percentages of the C₄ grass flora that use NAD-me, NADP-me and PEP-ck biochemical variants of C₄ photosynthesis for 15 sites in central Argentina.

rainfall and with both indices expressing the relationship between annual rainfall and temperature (Table 3). In contrast, the proportional representation of NAD-me-type grasses was greater than that of any other C₄ type in the dry part of the gradient (Table 2, Fig. 3). The percentage of NAD-me species showed a close negative relationship with rainfall and with

both Lang's and Emberger's indices. In both NADP-me and NAD-me types the pattern of variation was stronger when ground cover of grass species within the C₄ pathway was considered. NADP-me grasses apparently outcompeted NAD-me and PEP-ck types where precipitation was >600 mm year⁻¹, reaching cover values >60% with the exception of sites

Table 4 Correlations between C₄ grass floristic composition and climatic variables for 15 sites along a precipitation gradient in central Argentina. Grass variables are the percentage of C₄ grasses at a site that belong to a particular grass subfamily. For definitions of climatic variables see Table 1.

Variable	Chloridoideae (%)	Panicoideae (%)	Arundinoideae (%)
AR	-0.79***	0.86***	-0.33 NS
SR	-0.62**	0.68**	-0.29 NS
AAT	0.20 NS	-0.28 NS	0.25 NS
JAT	0.31 NS	-0.41 NS	0.32 NS
JUT	-0.23 NS	-0.20 NS	0.04 NS
L	-0.77***	0.85***	-0.36 NS
E	-0.72**	0.81***	-0.39 NS

** $P < 0.01$; *** $P < 0.001$.

6 and 8. In contrast, NAD-me cover was higher where annual rainfall was $< 500 \text{ mm year}^{-1}$ (Table 2, and see also Fig. 3 for % number of C₄ species). PEP-ck-type species were outnumbered and outcovered by both NADP-me- and NAD-me-type species along the whole range. PEP-ck species did not appear to have significant correlations with the measured climatic variables, except for a weak association with the temperature of the coldest month, even when cover data were included in the analysis (Table 3). Neither NADP-me nor NAD-me grasses showed significant associations with temperature variables (Table 3).

The distributions of the Chloridoideae and Panicoideae along the precipitation gradient followed opposite trends, with the former negatively ($r = -0.79$) and the latter positively ($r = 0.86$) correlated with annual rainfall (Fig. 3, Table 4). The subfamily Arundinoideae showed no significant correlations with any of the climatic variables.

DISCUSSION

C₃ vs. C₄ patterns

C₄ grass species dominated in at least 12 (considering the percentage of species) or 10 (considering relative cover) sites along the gradient. It appears, therefore, that C₄ grasses have certain advantages over C₃ species in most of the territory included in this study. Previous studies (Hattersley, 1983, 1992; Ehleringer & Monson, 1993) have shown that C₄ grasses tend to dominate where the wet season falls in the warmer summer months. The distribution pattern found for C₄ grasses in local floras from central Argentina was consistent with this. In central Argentina the percentage of C₄ grass species in local floras drops below 50% in the wetter eastern sites with winter and spring precipitation, enhancing the abundance of cool-season grasses that are largely C₃. The differences in the seasonal occurrence in precipitation constitute a plausible hypothesis to explain the proportional distribution of C₃/C₄ species in areas with relatively small differences in temperature. However, while this hypothesis is intuitively sound, Wan &

Sage (2001) have argued that the evidence supporting it is largely circumstantial. A plausible explanation is that of Ehleringer & Monson (1993), namely that the advantages of C₄ photosynthesis appear to have facilitated diversification and expansion into warm areas with relatively high summer rainfall.

A further pattern was that the percentage of C₄ grass species in local floras from central Argentina decreased as precipitation increased. C₃ grasses showed the opposite trend. As reported previously for different parts of the world, C₄ grasses are more likely to survive in arid and semi-arid environments than C₃ grasses, except in cold environments (Ellis *et al.*, 1980; Vogel *et al.*, 1986; Hattersley, 1992). Nevertheless, the pattern observed in this study largely supports the idea that C₄ grasses exhibit a wide tolerance to moisture conditions, from subhumid/mesic habitats ($> 600 \text{ mm annual rainfall}$) to extremely arid ones ($< 100 \text{ mm year}^{-1}$). A similar pattern has been reported by Ellis *et al.* (1980) in South West Africa, indicating that C₄ grasses may predominate in areas with widely differing precipitation regimes. An analysis of the distribution patterns of C₄ subtypes in relation to rainfall may provide further insights into this topic.

In agreement with Teeri & Stowe (1976) and Wan & Sage (2001), our results demonstrate that C₃/C₄ representations in local floras are more strongly correlated with summer temperatures than with annual average temperature. This observation is perhaps explained by the fact that in most of the sites along the gradient the grass flora is largely dormant during the winter. Hence, photosynthetic pathways are not correlated with winter temperatures. Within the temperature range of the study area, the percentages of both C₄ and C₃ grasses seem to be independent of the average temperature of the coldest month.

C₄ subtypes

The distributions of the NADP-me and NAD-me subtypes within local floras in central Argentina were significantly correlated with annual rainfall. The distribution pattern of the PEP-ck variant was not as clear as that of the other subtypes. No significant associations were observed between NADP-me and NAD-me variants and temperature variables. However, within the warmest sites where C₄ grasses dominated, malate-forming grasses attained their greatest representation in conditions of reduced water stress, while NAD-me species showed the opposite trend. These results confirm for the Neotropics the patterns observed in Namibia (Ellis *et al.*, 1980), in the Sinai, Negev and Judean deserts (Vogel *et al.*, 1986), in Australia (Hattersley, 1983, 1992), and in the United States (Taub, 2000), namely that NAD-me-type species predominate at the more arid extremes, whereas NADP-me-type species decrease with increasing aridity (Ehleringer & Monson, 1993). Within the range considered in this study, relative grass cover seems to be a better predictor of the relationships between photosynthetic subtypes and rainfall than does the percentage of species. In agreement with

Prendergast (1989), our results highlight the need to quantify the performance of photosynthetic C_4 variants in relation to climate using measurements such as cover or biomass.

The distribution patterns of C_4 species are largely related to the physiological advantages conferred by the higher physiological efficiency of C_4 photosynthesis under conditions of high light and temperature compared with C_3 species (Ghannoum *et al.*, 2001). Nevertheless, the underlying mechanisms of the differences in the distributions of the C_4 subtypes are unclear (Ghannoum *et al.*, 2001; Wan & Sage, 2001). Ghannoum *et al.* (2001) found no significant difference in water-use efficiency, CO_2 assimilation rate or stomatal conductance between NAD-me and NADP-me grasses. However, the analysis based on taxonomic identity of the species included in the experiments showed significant trends. In a later contribution, Ghannoum *et al.* (2002) showed that water stress enhances water-use efficiency in NAD-me grasses as compared with NADP-me species. More recently, Ghannoum *et al.* (2005) reported that NADP-me grasses achieved similar photosynthetic capacity to NAD-me species but with less leaf nitrogen content and Rubisco having higher turnover rate (k_{cat}). These results are consistent with those reported by Wan & Sage (2001), showing that NADP-me grasses may have a competitive advantage in conditions of higher water availability owing to their higher quantum yield. Alternatively, Taub (2000) found by means of partial correlation analysis that the association of grass subfamilies with annual rainfall was even stronger than that of the C_4 variants. The results presented by Taub (2000), together with our findings, suggest that different grasses may respond differently to climatic parameters such as rainfall. Such different responses could simply reflect the effect of divergent evolutionary histories (Kellogg *et al.*, 1999), and, as hypothesized by Prendergast (1989) and Taub (2000), the adaptation to different precipitation regimes could be associated with characteristics other than photosynthetic subtype.

CONCLUSIONS

The study area exhibits a rainfall gradient of more than 900 mm year^{-1} ; temperature exhibits a small increase towards the central and western parts of the rainfall gradient, but the trend is less clear. The distribution patterns of C_3 and C_4 grasses observed in local floras throughout this gradient are consistent with previous findings elsewhere. In all but three of the 15 sites studied, the C_4 type showed a higher percentage of species, while their relative cover was $> 50\%$ in 10 of the sites. This pattern is not unexpected in a predominantly warm-temperate to subtropical summer-rainfall area. Both the percentage and the relative cover of C_3 species decreased with rainfall and reached their maximum in sites with spring and winter rainfall.

Within the C_4 subtypes, the NADP-me species showed a higher proportional representation towards the mesic extreme of the moisture gradient, whereas the representation of the NAD-me species increased towards the more arid extreme. The relationship of PEP-ck species with climatic parameters in

central Argentina was less evident. The relationships between the C_4 subtypes and rainfall were stronger when relative cover was considered. This paper is the first to describe the pattern for the Neotropics and confirms previous findings in Africa, Asia, Australia and North America. The association of grass subfamilies Panicoideae and Chloridoideae with rainfall is as strong as that recorded for the NADP-me and NAD-me variants, respectively, suggesting that characteristics other than decarboxylation type may also be responsible for the geographic patterns described in this study.

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