



## Growth Responses of C<sub>4</sub> Grasses of Contrasting Origin to Elevated CO<sub>2</sub>

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Nine grass species representing three independent origins of the C<sub>4</sub> photosynthetic pathway were grown at ambient (350 ppm) and elevated (700 ppm) CO<sub>2</sub> and were harvested after flowering. *Setaria* and *Arundinella* are both members of the subfamily Panicoideae, and represent a single origin of the pathway. *Aristida* and *Stipagrostis* are sister genera in the subfamily Aristidoideae (formerly classified in subfamily Arundinoideae), and represent a second origin. *Sporobolus*, a member of the subfamily Chloridoideae, represents the third. By investigating two genera each within Panicoideae and Aristidoideae, we test the hypothesis that genera sharing the same origin of C<sub>4</sub> respond similarly. To explore variation among congeneric species, five species of *Setaria* were also examined to test the hypothesis that congeneric species have similar responses. Plant height and numbers of tillers, branches and inflorescences were measured, both over time and at final harvest. Biomass of roots, shoots, and inflorescences was also measured. Members of the Aristidoideae were generally significantly larger in elevated CO<sub>2</sub>, as indicated by measurements of biomass and plant height, whereas representatives of the Panicoideae varied considerably in their response. The two subfamilies differed significantly in their responses to elevated CO<sub>2</sub> and this effect outweighed any effect of CO<sub>2</sub> alone. *Sporobolus*, though equally distantly related to Panicoideae and Aristidoideae, had a CO<sub>2</sub> response similar to that of some panicoid species. Even within the genus *Setaria*, some species were significantly smaller at elevated than at ambient CO<sub>2</sub>, whereas others were larger. This may reflect diversity in internal regulation rather than acclimation or changes in source-sink allocation of carbon. The variation complicates any prediction of responses of C<sub>4</sub> plants to future atmospheric change. Comparison of closely related species, however, may well lead to intriguing new insights into how regulatory pathways of CO<sub>2</sub> assimilation are modified during evolution.

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**Key words:** *Aristida*, *Arundinella*, C<sub>4</sub> photosynthesis, CO<sub>2</sub>, global change, Poaceae, *Setaria*, *Sporobolus*, *Stipagrostis*.

### INTRODUCTION

C<sub>4</sub> photosynthesis, which makes CO<sub>2</sub> fixation more efficient, is an extension of the standard C<sub>3</sub> pathway (Hatch, 1976; Edwards and Huber, 1981; Edwards and Walker, 1983). C<sub>4</sub> plants use phosphoenolpyruvate carboxylase (PEPC) in the mesophyll to catalyze the addition of atmospheric CO<sub>2</sub> to the 3-carbon phosphoenolpyruvate (PEP) to create a 4-carbon compound (oxaloacetate or malate). The 4-carbon compound is then transported to the cells surrounding the vascular bundle where the newly fixed carbon is released and then attached to ribulose 1,5-bisphosphate (RuBP) by RuBP carboxylase/oxygenase (Rubisco). The latter reaction is the first step of the conventional C<sub>3</sub> pathway. C<sub>4</sub> plants thus sequester their entire C<sub>3</sub> pathway in bundle sheath cells, and create an additional, novel carbon fixation pathway in the mesophyll.

Although the C<sub>4</sub> pathway is apparently complex genetically, it has arisen multiple times in the angiosperms. It occurs in over 1000 species in 19 families (Sage and Monson, 1999) and has apparently developed several times in the grasses (Poaceae) alone (Fig. 1).

This paper examines responses of several related C<sub>4</sub> grass taxa to varied levels of atmospheric carbon dioxide which is projected to affect the physiology and community ecology of C<sub>4</sub> plants in the future as it increases due to anthropogenic inputs (Wray and Strain, 1986; Bazzaz *et al.*, 1989; Bazzaz, 1990; Drake, 1992). We test the null hypothesis that, among closely related but independently derived C<sub>4</sub> lineages, response to elevated CO<sub>2</sub> is largely the same. Therefore, a pair of genera was chosen within the subfamily Panicoideae, a second pair within the subfamily Aristidoideae, and a single representative of subfamily Chloridoideae for comparison. (Members of a second chloridoid genus failed to establish.) This sampling strategy also allowed us to test whether genera within a subfamily differ in their CO<sub>2</sub> response.

Similarly, we hypothesized that congeneric species would exhibit similar responses to elevated CO<sub>2</sub>. This hypothesis was based on the idea that closely related species are more similar to each other in their responses to elevated CO<sub>2</sub> than they are to more distantly related taxa. To test this hypothesis, five species in the genus *Setaria* were examined.

C<sub>4</sub> grasses are not expected *a priori* to be responsive to rising CO<sub>2</sub> levels, since their rate of photosynthesis is assumed not to be carbon-limited (Percy and Björkman,

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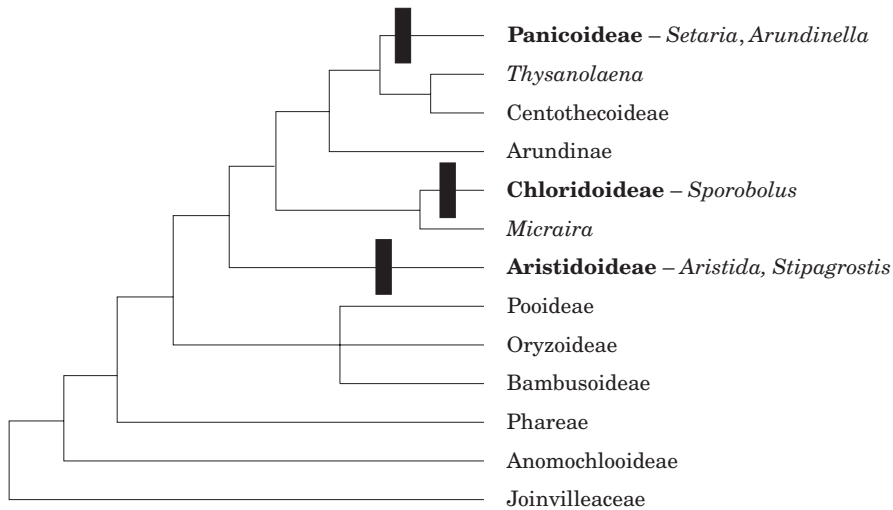


FIG. 1. Phylogeny of the grass family, based on sequences of *ndhF* (Clark *et al.*, 1995), showing the relationship of genera in this study. Taxa that are C<sub>4</sub> are in bold, and origins of C<sub>4</sub> photosynthesis are shown with a broad line.

1983). That is, if CO<sub>2</sub> fixation by Rubisco is not a rate-limiting step in C<sub>4</sub> photosynthesis, then raising the partial pressure of CO<sub>2</sub> will not stimulate carbon uptake. Some studies have confirmed this assumption (see, for example, the C<sub>4</sub> species *Paspalum dilatatum*, *Paspalum conjugatum*, *Spartina patens*, *Distichlis spicata* and *Tridens flavus*; Curtis *et al.*, 1989; Marks and Clay, 1990; Ziska *et al.*, 1991; Clark *et al.*, 1997). Other studies, however, report wide variation in the responsiveness of C<sub>4</sub> plants to CO<sub>2</sub> levels. Some C<sub>4</sub> grasses have shown significant CO<sub>2</sub>-related enhancements in growth (Poorter, 1993; LeCain and Morgan, 1998), but data on *Sorghum bicolor* (Mauney, Fry and Guinn, 1978; Carter and Peterson, 1983; Morison and Gifford, 1984) and *Zea mays* (Wong, 1979; Morison and Gifford, 1984; King and Greer, 1986; Hunt *et al.*, 1991) are contradictory. Some of the reported variation may reflect interaction with other environmental factors such as light (Sionit and Patterson, 1984; Ghannoum *et al.*, 1997), temperature (Read and Morgan, 1996), nitrogen (Ghannoum and Conroy, 1998), or salt stress (Bowman and Strain, 1987). Wray and Strain (1986), however, found no interaction between CO<sub>2</sub> and drought (which should have a physiological effect similar to salt stress) in *Andropogon virginicus*.

Wand *et al.* (1999) have conducted a meta-analysis including all available studies of wild C<sub>4</sub> and C<sub>3</sub> grasses and their responses to elevated CO<sub>2</sub>. Their results show that C<sub>4</sub> grasses do respond to elevated CO<sub>2</sub> with significant increases in biomass and in leaf area.

To what extent can one generalize about ecological and physiological responses of C<sub>4</sub> plants? It is tempting to assume that C<sub>4</sub> plants, and in particular C<sub>4</sub> grasses, are more similar to each other than they are to C<sub>3</sub> plants. This is only true if all origins of the C<sub>4</sub> pathway are truly convergent, i.e. multiple 'machines' all generating the same sort of output. Structural and biochemical differences occur among C<sub>4</sub> photosynthetic systems, despite broad similarities in photosynthetic mechanisms. These differences may reflect divergent evolutionary histories, and may also influence the

physiological responses of different C<sub>4</sub> lineages to past and future selection pressures. For example, even in a single family such as the Poaceae, taxa within C<sub>4</sub> lineages differ in anatomy, leaf histology and in localization of several key photosynthetic enzymes (Sinha and Kellogg, 1996). One class of C<sub>4</sub> plants uses NADP as a co-factor (NADP-ME) in decarboxylation and another class uses NAD (NAD-ME). A few species also use PEP-carboxykinase in addition to NAD-ME (Hattersley and Watson, 1992). Although NAD-ME-type and NADP-ME-type grasses may respond differently to various environmental parameters, such different responses could simply reflect the effect of divergent evolutionary histories (D. Taub and M. Lerdau, pers. comm.).

If the grasses studied here do not respond in accordance with their phylogenetic relationships, then it is worth looking more closely at their ecological preferences or differences in life history such as annual *vs.* perennial habit. The C<sub>4</sub> pathway is generally interpreted as an adaptation to hot, dry environments (Ehleringer and Monson, 1993). The proportion of C<sub>4</sub> plants in a given flora correlates well with mean July temperature—higher temperatures are associated with more C<sub>4</sub> species (Hattersley, 1983). However, different C<sub>4</sub> lineages are known to vary in their environmental preferences; their present-day responses may be a legacy of differing selective regimes. For example, distribution of C<sub>4</sub> monocots is correlated with temperature, whereas distribution of C<sub>4</sub> dicots may more closely reflect aridity (Ehleringer, Cerling and Helliker, 1997). Similarly, chloridoid grass species are more often found in hot dry habitats of Australia and southern Africa, whereas panicoid grasses occur more commonly in more mesic environments (Hattersley, 1983, 1992).

None of the numerous studies of responses of C<sub>4</sub> grasses to elevated CO<sub>2</sub> considered the effect of phylogenetic history on physiological responses. Comparative studies of C<sub>4</sub> lineages can elucidate subtle differences in photosynthetic pathways and their ramifications for plant growth, fitness

and evolution, especially in changing climates. Here, we describe the response in growth, carbon allocation and reproduction by three lineages of C<sub>4</sub> grasses grown in ambient and elevated atmospheric CO<sub>2</sub>.

## MATERIALS AND METHODS

### *Taxa*

One species each of *Arundinella*, *Aristida*, *Stipagrostis* and *Sporobolus* (Table 1) was investigated; in addition, five species of *Setaria* were studied to explore variation among congeneric species. *Setaria* and *Arundinella* are members of the subfamily Panicoideae, *Aristida* and *Stipagrostis* are sister genera in the subfamily Aristidoideae, and *Sporobolus* is a representative of the Chloridoideae. *Eragrostis*, another member of the Chloridoideae, was investigated, but too few seedlings survived for adequate statistical replication. The three groups represent independent derivations of the C<sub>4</sub> photosynthetic pathway (Fig. 1; Barker, Linder and Harley, 1995; Clark, Zhang and Wendel, 1995; Soreng and Davis, 1998). All are NADP-ME subtypes, except for *Sporobolus*, which is NAD-ME, and *Stipagrostis*, which has not been biochemically typed, but is almost certainly NAD-ME (Hattersley, 1992). The species vary in biogeographic distribution, habitat preferences and life history (Table 1).

### *Growth conditions and experimental design*

Seeds were sown in two nursery flats per species in the Harvard University controlled CO<sub>2</sub> glasshouse facility, Cambridge, Massachusetts, USA. The glasshouse contains six modules, three of which are maintained at ambient CO<sub>2</sub> (350 ppm), the others at double ambient levels (700 ppm). Soon after germination in late March 1996, 30 seedlings of each species were transplanted to individual 20-cm-diameter nursery pots containing a peat/soil/vermiculite potting mix. These were divided into sets of five plants per species and distributed among the two CO<sub>2</sub> treatments in the six glasshouse modules. The final design for each species was thus five plants × three modules × two CO<sub>2</sub> concentrations. Sunlight levels ranged from 600–1300 μmol m<sup>-2</sup> s<sup>-1</sup> photosynthetically active radiation. Diurnal/nocturnal temperatures were 26/19 °C and relative humidities ranged from 75 to 95%. Plants were watered daily and were fertilized weekly with 0.25 g l<sup>-1</sup> Peters NPK 20:20:20.

### *Growth parameters*

Plant height (to the top of the longest leaf or the tallest inflorescence, whichever was taller), and numbers of tillers, branches and inflorescences per plant were recorded several times during the growing season. Because formation of tillers (branches at or below ground level) and of culm branches may both be the result of a single physiological response, the two measurements were combined into a measure of total meristems.

Plants were harvested when seeds began to ripen but before plant senescence, to quantify biomass allocation to

reproduction. The time from planting to seed maturation differed among species. Harvests occurred at specific life stages rather than standard absolute times. Plants were harvested and divided into inflorescences, above-ground vegetative material (stems plus leaves), and roots. Plant material was dried at 70 °C for a minimum of 1 week, and then weighed to the nearest 0.01 g. Above-ground dry biomass was calculated as stems plus leaves plus inflorescences. Root:shoot ratio was root dry biomass divided by above-ground dry biomass.

### *Statistical analyses*

Raw data for all variables were graphed in scatter plots to check for outliers. Four plants (1.7% of the total) were identified that were chlorotic throughout growth and substantially smaller than conspecifics. These individuals were eliminated from the sample on the grounds that they had grown abnormally poorly and were not representative of the population. (Their inclusion in statistical analyses increased variance but did not alter treatment trends.) All statistical comparisons were performed using Systat for Windows version 5.05, after log-transformation (as needed) to eliminate heteroscedasticity of the data. Normal probability plots and residual plots were inspected to ensure that the data met the assumptions of ANOVA. Single harvest measures such as plant mass were examined with ANOVA, while non-destructive measures of height growth, branch and inflorescence production, and tillering were analysed for separate species using a repeated-measures design. Root biomass, inflorescence biomass, and stem+leaf biomass were found to be significantly correlated (Pearson's product-moment), and were therefore examined for responses to CO<sub>2</sub> using multivariate analysis of variance (MANOVA). To investigate whether related genera within subfamilies responded similarly to CO<sub>2</sub>, a summary nested model was tested, comparing *Arundinella* and pooled *Setaria* species (nested within Panicoideae) to *Aristida* and *Stipagrostis* (nested within Aristidoideae). Module identity did not contribute to the overall variance explained by these models, so was omitted from final among-species analyses.

To examine similarities among taxa in correlated CO<sub>2</sub> responses, response ratios (mean value of a variable in high CO<sub>2</sub>/mean value in ambient CO<sub>2</sub>) were computed on twelve growth, harvest mass and mortality variables. The response ratios were then assembled into a matrix with species arrayed as rows, and principal components analysis (PCA) was performed using the 'princomp' function of S-Plus (version 3.3) for Windows.

Preliminary PCA analyses indicated a strong correlation among all measurements of biomass, suggesting that they all measured a single underlying process. Only total biomass and inflorescence biomass were included therefore in the final PCA. Construction of PCA using response ratios (whose underlying distributions may violate assumptions of parametric statistics) can be problematic (Jasienski and Bazzaz, 1999). We use PCA here as graphical exploratory data analysis only to illustrate distribution of species in character space and to visualize trends that are borne out in

TABLE 1. Species included in this study and their phylogenetic placement

Species	C <sub>4</sub> subtype	Range	Life history	Voucher specimen
Subfamily Panicoideae, tribe Arundinelleae				
<i>Arundinella hirta</i> L.	NADP-ME	Dry grasslands, Asia	Perennial	Kellogg PI263693
Subfamily Panicoideae, tribe Paniceae				
<i>Setaria italica</i> (L.) Beauv.	NADP-ME	Cosmopolitan, US and Eurasia	Annual	Kellogg V33
<i>Setaria macrostachya</i> Kunth	NADP-ME	Southern Texas, Arizona, Mexico	Perennial	Kellogg V40
<i>Setaria pumila</i> (Poir.) Roem. & Schult.	NADP-ME	Disturbed dry tropical habitats	Annual	Kellogg V39
<i>Setaria faberi</i> Hermann	NADP-ME	China and midwestern US; weedy	Annual	Kellogg V32
<i>Setaria lutescens</i> (Weigel) F. T. Hubb.	NADP-ME	Cosmopolitan north temperate weed	Annual	Kellogg V41
Subfamily Chloridoideae				
<i>Sporobolus cryptandrus</i> (Torr.) A. Gray	NAD-ME	Widespread, dry sites, northern US and Canada	Perennial	Kellogg V29
Subfamily Aristidoideae				
<i>Aristida oligantha</i> Michx.	NADP-ME	Widespread, dry sites, northern US and Canada	Annual	Kellogg V38
<i>Stipagrostis hirtigluma</i> (Trin. & Rupr.) De Winter	NAD-ME	Southern Africa, dry savanna, karoo	Annual	Kellogg V31

Vouchers are deposited at the Harvard University Herbaria (GH). Data on photosynthetic pathway from Hattersley and Watson (1992). Information on range and life history from Rominger (1962), Clayton and Renvoize (1982), Cronquist *et al.* (1977), Gibbs Russell *et al.* (1990) and Koyama (1987).

the explicit statistical analyses computed to test phylogenetically-based hypotheses.

## RESULTS

Subfamilies responded significantly differently to high CO<sub>2</sub>. The Panicoideae (*Setaria* and *Arundinella*) and the Aristidoideae (*Aristida* and *Stipagrostis*) differed significantly in all parameters except plant height and total biomass (Table 2). The effect of CO<sub>2</sub> alone, however, was not significant except for total branch number. In terms of growth parameters, the Aristidoideae produced more meristems at elevated CO<sub>2</sub>, whereas the Panicoideae generally branched and tillered less (Fig. 2), as did the one chloridoid species. The two subfamilies differed significantly in their

average relative growth rate. Numbers of inflorescences were unaffected by elevated CO<sub>2</sub> in the Aristidoideae, but were significantly affected in four of the six panicoid species (two increased and two decreased); the chloridoid species showed a significant reduction in inflorescence number. The Aristidoideae responded positively to elevated CO<sub>2</sub> in terms of biomass across all plant compartments. By contrast, most panicoid species responded negatively, as did the chloridoid species. These generalizations, however, obscure many differences between genera within subfamilies.

The differences among genera far outweighed the differences between CO<sub>2</sub> treatments or among subfamilies (Table 2; Fig. 2). In terms of plant height, *Aristida oligantha* was significantly taller in high CO<sub>2</sub>, but branching was unaffected, whereas *Stipagrostis* exhibited the opposite

TABLE 2. Nested ANOVA for the following variables: CO<sub>2</sub>, subfamily, genus nested within subfamily, subfamily × CO<sub>2</sub>

Source	Final height		Final tiller number		Final branch number		Final inflorescence number	
	F	P	F	P	F	P	F	P
CO <sub>2</sub>	0.757	0.385	1.978	0.161	20.116	< 0.0001	0.008	0.930
Subfamily	0.790	0.375	46.770	< 0.0001	386.898	< 0.0001	57.009	< 0.0001
Genus within subfamily	9.301	< 0.0001	16.164	< 0.0001	59.408	< 0.0001	40.032	< 0.0001
Subfamily × CO <sub>2</sub>	1.187	0.277	25.394	< 0.0001	15.699	< 0.0001	0.088	0.757
Source	Total plant mass		Log root:shoot ratio		Mean relative growth			
	F	P	F	P	F	P		
CO <sub>2</sub>	0.226	0.635	0.144	0.705	1.690	0.195		
Subfamily	3.601	0.059	155.375	< 0.0001	12.109	0.001		
Genus within subfamily	9.401	< 0.0001	120.279	< 0.0001	127.055	< 0.0001		
Subfamily × CO <sub>2</sub>	5.734	0.017	0.939	0.334	0.097	0.755		

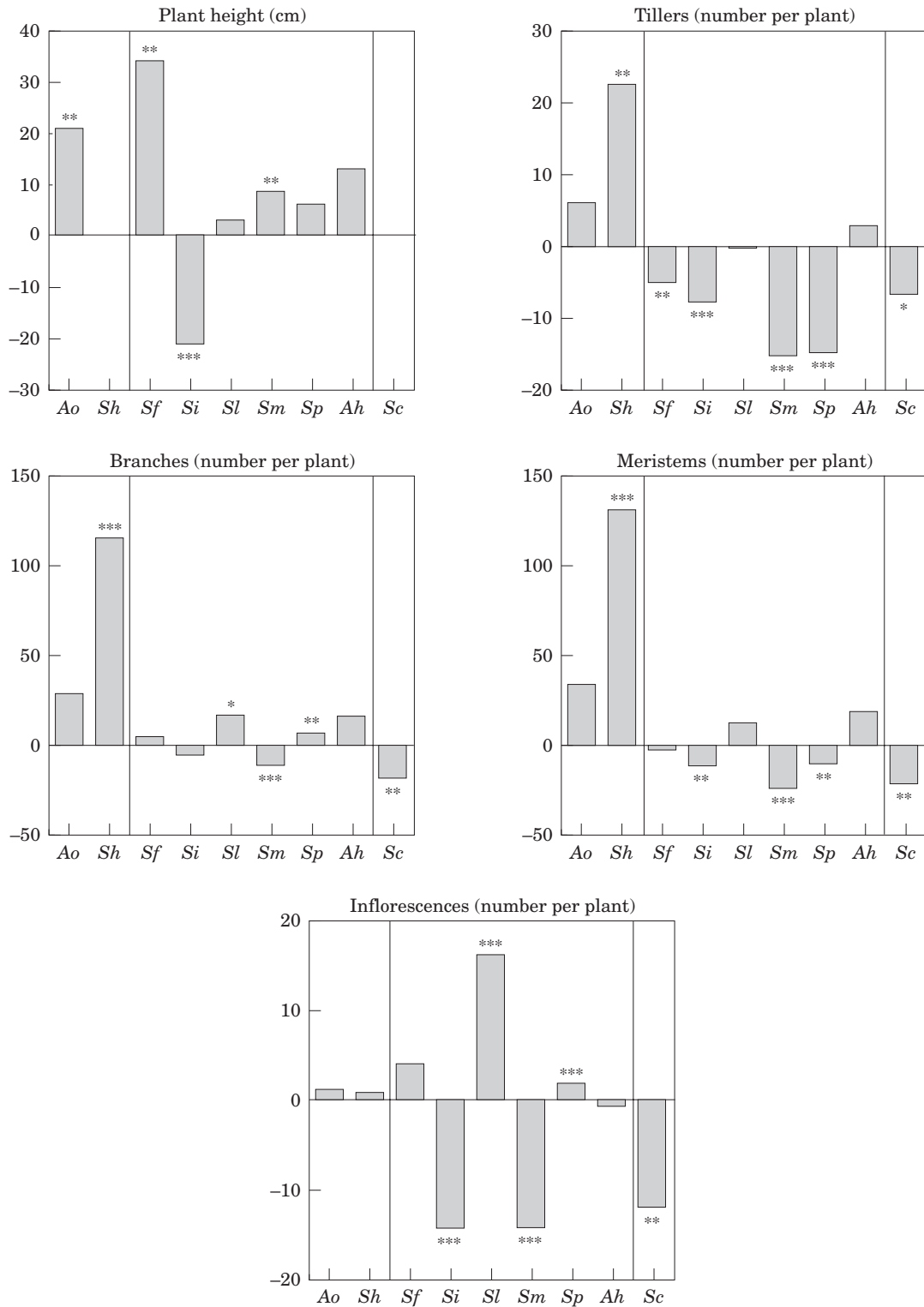


FIG. 2. Difference in number of tillers, inflorescences and meristems (= tillers+branches), and plant height (cm) for elevated CO<sub>2</sub> minus ambient CO<sub>2</sub>. \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ . Vertical lines delimit separate C<sub>4</sub> lineages. Ao, *Aristida oligantha*; Sh, *Stipagrostis hirtiglumis*; Sf, *Setaria faberi*; Si, *S. italica*; Sl, *S. lutescens*; Sm, *S. macrostachya*; Sp, *S. pumila*; Ah, *Arundinella hirta*; Sc, *Sporobolus cryptandrus*.

response (Fig. 2). Within subfamilies there were significant differences between genera in relative growth rate (Table 2). Inflorescence production was unaffected in either aristidoid genus. Both genera of Aristidoideae showed enhanced

biomass at elevated CO<sub>2</sub>, but enhancement was significant only in *Stipagrostis* (Fig. 3). In the Panicoideae, plant height, meristem production and inflorescence number were unaffected by elevated CO<sub>2</sub> in *Arundinella*, but for these

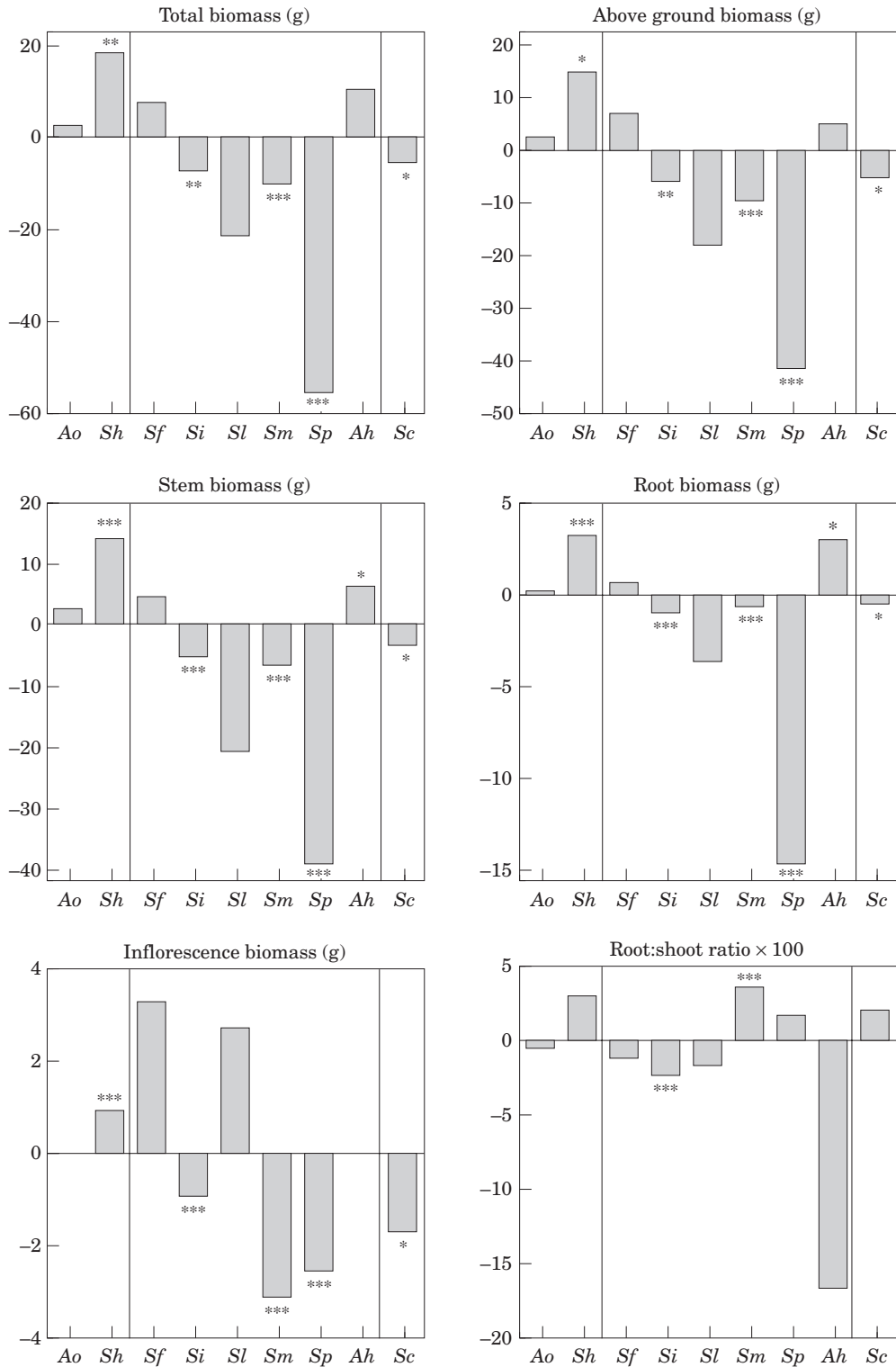


FIG. 3. Difference in total, above-ground, stem, root and inflorescence biomass, and root:shoot ratio (× 100) for elevated CO<sub>2</sub> minus ambient CO<sub>2</sub>. Symbols as for Fig. 2.

same parameters, the species of *Setaria* were sensitive to CO<sub>2</sub> level (Fig. 2). *Arundinella* exhibited consistently positive enhancements of biomass, while elevated CO<sub>2</sub> generally resulted in reduced biomass of species of *Setaria*.

Species within the genus *Setaria* respond significantly differently to high CO<sub>2</sub>. Considering *Setaria* alone, there was a significant species × CO<sub>2</sub> interaction for all parameters. Four of the five *Setaria* species (all except *S.*

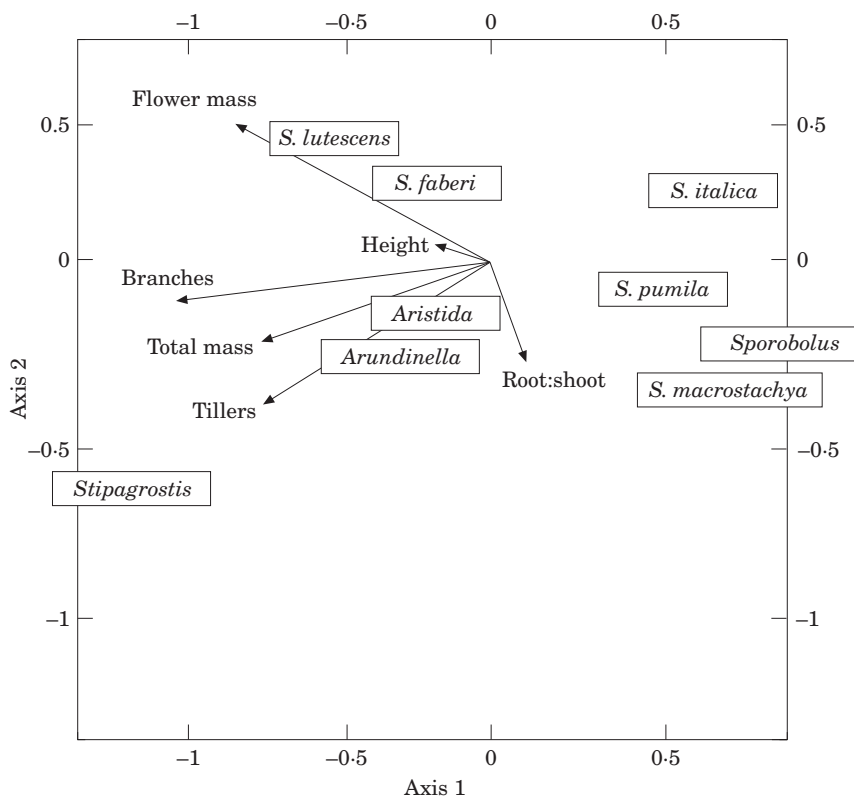


FIG. 4. Principal component analysis showing loading of characters on the first two component axes. First two axes explain 80% of the total variance. Taxa are plotted in character space.

*lutescens*) had fewer tillers in high CO<sub>2</sub> beginning at 12 d after first measurement. The difference in tiller number between CO<sub>2</sub> treatments increased over time. *Setaria lutescens* and *S. pumila* produced more branches in high CO<sub>2</sub>. In *S. italica*, numbers of branches were higher earlier in development, but then lower by the time of harvesting. *Setaria macrostachya* branched very little during development, but by the time of harvest had fewer branches in high CO<sub>2</sub>. *Setaria italica*, *S. macrostachya* and *S. pumila* all had fewer total meristems in high CO<sub>2</sub>, but the other species exhibited no difference (Fig. 2). *Setaria macrostachya* and *S. italica* had fewer inflorescences in high CO<sub>2</sub>, whereas *S. lutescens* and *S. pumila* had more. There was no significant difference for *S. faberi*.

Biomass in all compartments declined in *Setaria italica*, *S. macrostachya* and *S. pumila*, but did not change in *S. faberi* or *S. lutescens* (Fig. 3). This is in contrast to the results of Carlson and Bazzaz (1980, 1982) and Sionit and Patterson (1984, 1985), who found that biomass in *S. faberi* and *S. lutescens* increased at elevated CO<sub>2</sub>. Root:shoot ratio significantly decreased in *Setaria italica* and increased in *S. macrostachya*.

Results of exploratory PCA of branch number, tiller number, plant height, total biomass, inflorescence biomass, and root:shoot ratio were similar to the results of other analyses (Fig. 4). The first axis explained 63% of the total variance and the second axis an additional 17%, implying that the two-dimensional plot is a reasonable reflection of

the distribution of species in character space. Congeneric species differed in their response to elevated CO<sub>2</sub>, as shown by the distance between *Setaria faberi* and *S. lutescens* on the one hand and *S. italica*, *S. pumila* and *S. macrostachya* on the other. The chloridoid species *Sporobolus cryptandrus* was most similar to the panicoid *S. macrostachya*, and *Stipagrostis* was quite different from its close relative *Aristida*. The correlation among meristem number, tiller number and total biomass is apparent from the similar direction of the vectors for each. This suggests that they may reflect similar underlying processes; for example, increases in total biomass may be caused by increases in meristem number.

## DISCUSSION

### Variation among C<sub>4</sub> plants

Not all C<sub>4</sub> grasses respond in the same way to elevated CO<sub>2</sub>. Thus it is difficult to generalize about the potential response of C<sub>4</sub> plants to climate change and other environmental parameters. This study mirrors the many complex and contradictory responses reported in previous studies.

Species identity is an important factor in CO<sub>2</sub> response, even among congeners. It is clear from comparative studies in high CO<sub>2</sub> that even closely related plant species do not necessarily use carbon in equivalent ways (Farnsworth and Bazzaz, 1995). The results of this study show that variation among species within a genus in response to elevated CO<sub>2</sub> is

as great as variation between genera within a subfamily and between subfamilies within a family. What factors might explain the variation in response to elevated CO<sub>2</sub>? Although C<sub>4</sub> subtype might contribute to the differences in CO<sub>2</sub> response, the variation documented here and reported in the literature does not correlate with C<sub>4</sub> subtype. Because *Sporobolus cryptandrus* was the only known NAD-ME type species included in this study, we could not test differences in response between C<sub>4</sub> subtypes. *Sporobolus*, however, is similar to several of the *Setaria* species (which are all NADP-ME) in its response to high CO<sub>2</sub> (Fig. 4). Furthermore, the amount of variation among the NADP-ME types is so high that differences between NAD-ME and NADP-ME species would be difficult to detect.

Members of the genus *Stipagrostis* have not yet been biochemically typed, but on the basis of leaf anatomy, Hattersley and Watson (1992) posited that it is of the NAD-ME subtype. *Stipagrostis* was quite different from all the other species, including the NAD-ME *Sporobolus*, in that all growth and biomass measurements were significantly higher at high CO<sub>2</sub>; this was unexpected for a C<sub>4</sub> grass, although it would not be surprising in a C<sub>3</sub> species. Its unusual response to elevated CO<sub>2</sub> suggests that further anatomical and physiological study may be warranted.

The estimates of growth enhancement reported by Poorter (1993) and by LeCain and Morgan (1998) were not significantly different for the NAD-ME-type and NADP-ME-type grasses. Work of Taub and Lerda (unpubl. res.) on nitrogen and drought responses of the two subtypes found that the C<sub>4</sub> subtype does not correlate with physiological response, but correlates instead with subfamilial classification. Chloridoid species are significantly different from panicoid species, independent of subtype. In other words, the differences are explained by shared phylogenetic history.

Although biogeographic distribution, habitat preferences and life history might be expected to influence response to elevated CO<sub>2</sub>, we did not find an obvious or consistent relationship between any growth parameters and these ecological characteristics. For example, *Arundinella hirta*, *Sporobolus cryptandrus* and *Setaria macrostachya* are all perennials (Table 1), but the latter two were significantly smaller in elevated CO<sub>2</sub> than in ambient conditions, whereas *Arundinella* was unaffected. *Sporobolus cryptandrus* and *Aristida oligantha* share a similar distribution in the northern US and Canada, but differ in their responses to elevated CO<sub>2</sub>. The widespread weeds (*Setaria* species, *Aristida*) are no more similar to each other in their response than they are to the species of undisturbed habitats (*Arundinella hirta* and *Stipagrostis hirtigluma*).

Some species in this study were adversely affected by elevated CO<sub>2</sub> levels, confirming results reported in the literature for *Sorghum bicolor* and *Andropogon glomeratus* (Carter and Peterson, 1983; Bowman and Strain, 1987). This result was somewhat surprising. Acclimation to high CO<sub>2</sub> in C<sub>3</sub> plants is a common phenomenon, but generally does not result in plants that are smaller than those at ambient CO<sub>2</sub>. Pot size is known to affect response to CO<sub>2</sub>, as do shade and watering (Arp, 1991; Thomas and Strain, 1991). However, these generally result in plants that are the

same size at both CO<sub>2</sub> concentrations. Similarly if N is limiting, then high CO<sub>2</sub> plants may be prevented from becoming larger, but they should not necessarily become significantly smaller.

Decreased size may be the result of differential carbon allocation and increased root turnover. If high CO<sub>2</sub> increases allocation to roots, with a commensurate decrease in above-ground biomass, and if root turnover simultaneously increases, then the result could be a smaller plant. There were no significant changes in root:shoot ratio, however, in any of the plants in this study. This means that if root turnover had increased in these plants, it would have to match precisely the increase in below-ground biomass. Such a precise match seems unlikely, and therefore increased root turnover is unlikely to be a factor in the results presented here.

It is also possible that trace contaminants in the added CO<sub>2</sub> might lead to reduced growth in some species. This hypothesis would require that some of the species be more sensitive than others to the putative contaminant. While we cannot rule out this possibility, we also have no evidence in favour of it.

Decreased size may also be caused by internal regulatory changes. Both carboxylases—Rubisco and PEPC—are adversely affected by their end products. Expression of small subunit genes of Rubisco is known to be inhibited by 6-carbon sugars (Sheen, 1994), and PEPC catalysis is inhibited by malate (Chollet, Vidal and O'Leary, 1996). If some plants were unable to process one of these end products rapidly enough, then the substances would accumulate and inhibit carbon fixation. In support of this hypothesis, most of the plants that responded negatively (or not at all) to high CO<sub>2</sub> showed reduced relative growth rates throughout the duration of the experiment. This indicates that growth rate responses occurred while the plants were still young, well before any pot size effects would have been observed. If this hypothesis is correct, however, the regulation must be more complex than simple end-product inhibition, which would presumably become stronger as growth proceeds and sink strength declines.

#### Implications for future work

This study has important implications for future studies on the effects of elevated CO<sub>2</sub>, as well as for research on the evolution of C<sub>4</sub> photosynthetic pathways and other complex adaptations with multiple origins. Our results show considerable variability both among taxa that share a convergent occurrence of the C<sub>4</sub> photosynthetic pathway, and among congeneric species that represent a single origin of the C<sub>4</sub> pathway. These findings prevent steady generalizations about past or projected responses of all C<sub>4</sub> plants to changes in atmospheric CO<sub>2</sub> concentrations. Such differences in growth response to CO<sub>2</sub> level may reveal much about how the physiology of different C<sub>4</sub> pathways constrains and enhances carbon uptake and conversion.

Growth rate and biomass accumulation integrate all aspects of carbon assimilation and allocation into a single set of parameters. More and more physiological and genetic data show that 6-carbon sugars—the primary products of

photosynthesis—they themselves regulate many disparate cellular processes, so that the plant is exquisitely sensitive to internal carbon levels. These internal regulatory networks are apparently complex and, as yet, poorly understood (Sheen, 1994; Koch, 1996). This study suggests that changes in overall carbon availability have different effects depending on the genetic background of the plants. This is consistent with non-linear, highly complex responses to changing amounts of intracellular carbon. Furthermore, it appears that the strength of interactions among regulatory parameters may differ from species to species.

For those wanting to predict response to global changes in CO<sub>2</sub>, this conclusion is concerning because it implies remarkably non-uniform responses in plants. For those seeking to understand carbon metabolism, however, our results suggest that comparison of closely related species may reveal multiple disparate regulatory pathways.

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