

Review Article

Responses of wild C4 and C3 grass (Poaceae) species to elevated atmospheric CO₂ concentration: a meta-analytic test of current theories and perceptions

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Abstract

C4 plants contribute ≈20% of global gross primary productivity, and uncertainties regarding their responses to rising atmospheric CO₂ concentrations may limit predictions of future global change impacts on C4-dominated ecosystems. These uncertainties have not yet been considered rigorously due to expectations of C4 low responsiveness based on photosynthetic theory and early experiments. We carried out a literature review (1980–97) and meta-analysis in order to identify emerging patterns of C4 grass responses to elevated CO₂, as compared with those of C3 grasses. The focus was on nondomesticated Poaceae alone, to the exclusion of C4 dicotyledonous and C4 crop species. This provides a clear test, controlled for genotypic variability at family level, of differences between the CO₂-responsiveness of these functional types. Eleven responses were considered, ranging from physiological behaviour at the leaf level to carbon allocation patterns at the whole plant level. Results were also assessed in the context of environmental stress conditions (light, temperature, water and nutrient stress), and experimental growing conditions (pot size, experimental duration and fumigation method).

Both C4 and C3 species increased total biomass significantly in elevated CO₂, by 33% and 44%, respectively. Differing tendencies between types in shoot structural response were revealed: C3 species showed a greater increase in tillering, whereas C4 species showed a greater increase in leaf area in elevated CO₂. At the leaf level, significant stomatal closure and increased leaf water use efficiency were confirmed in both types, and higher carbon assimilation rates were found in both C3 and C4 species (33% and 25%, respectively). Environmental stress did not alter the C4 CO₂-response, except for the loss of a significant positive CO₂-response for above-ground biomass and leaf area under water stress. In C3 species, stimulation of carbon assimilation rate was reduced by stress (overall), and nutrient stress tended to reduce the mean biomass response to elevated CO₂. Leaf carbohydrate status increased and leaf nitrogen concentration decreased significantly in elevated CO₂ only in C3 species.

We conclude that the relative responses of the C4 and C3 photosynthetic types to elevated CO₂ concur only to some extent with expectations based on photosynthetic theory. The significant positive responses of C4 grass species at both the leaf and the whole plant level demand a re-evaluation of the assumption of low responsiveness in C4 plants at both levels, and not only with regard to water relations. The combined shoot structural and water use efficiency responses of these functional types will have consequential implications for the water balance of important catchments and range-

lands throughout the world, especially in semiarid subtropical and temperate regions. It may be premature to predict that C4 grass species will lose their competitive advantage over C3 grass species in elevated CO₂.

Keywords: C4 grasses, C3 grasses, climate change, elevated CO₂, gas exchange and growth responses, meta-analysis

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Introduction

The effects of atmospheric CO₂ enrichment have been studied in great detail for agricultural crops (Cure & Acock 1986), trees (Ceulemans & Mousseau 1994), and other plant types (Bazzaz 1990; Poorter 1993; Idso & Idso 1994). The great majority of these studies have been carried out on C3 species. Much of the early research into CO₂-responses of C4 species focused on weedy and old-field dicotyledonous (dicot) species (e.g. *Amaranthus* sp., Bazzaz & Carlson 1984), or important planted C4 crop species (e.g. *Zea mays* and *Sorghum* sp., Morison & Gifford 1984). Despite the fact that about half of the world's grass species possess the C4 photosynthetic pathway, fewer studies have tested the responses of wild temperate C4 grasses to elevated CO₂, and only a handful have used tropical C4 grasses. These plants account for ≈18% of the total global productivity, mainly due to the extensive grasslands and savannas of the tropics (Ehleringer *et al.* 1997), but they also play an important role in mixed temperate grasslands such as the North American prairies. Lloyd & Farquhar (1994), using a modelling approach based on ¹³C discrimination, estimated a contribution of 21% by C4 plants to global gross primary productivity (GPP) under current atmospheric conditions. Any changes in C4 productivity driven by CO₂ and other climatic perturbations will, consequently, have a substantial impact on global GPP.

By far the greatest proportion of C4 species are monocotyledonous (monocot), whereas C4 dicots are relatively uncommon, both in terms of species representation and abundance (Ehleringer *et al.* 1997). Many C4 dicots are noxious weeds and old-field invaders, and gain importance only in disturbed sites. Thus, studies using C4 dicots and bred crops may not represent the potential CO₂-responsiveness of natural, relatively undisturbed ecosystems with a significant C4 monocot component, such as prairies, tropical grasslands, and savannas. Nevertheless, results obtained from many of these studies, and from the first field-based study of a C4-containing ecosystem, a salt marsh on Chesapeake Bay (Curtis *et al.* 1989), appeared to confirm the theory that C4 plants should not show significant growth responses to elevated CO₂, due to their CO₂-concentrating mechanism in the bundle sheath cells (Osmond *et al.* 1982; Percy & Ehleringer 1984; Bowes 1993). This mechanism increases

the effective concentration of CO₂ at the site of carboxylation, thereby masking photorespiration and apparently ensuring saturation of photosynthesis at current atmospheric CO₂ concentrations. It follows, in theory, that C4 plants should not benefit from increased atmospheric CO₂ availability, and may suffer reduced competitive advantage over C3 species (Bazzaz 1990; Bowes 1993; Ehleringer & Monson 1993). As a result of this common perception, the potential contribution of C4-dominated ecosystems to the global carbon budget in a future high-CO₂ environment, especially in the highly productive tropics, has been largely discounted or ignored.

It is now becoming increasingly clear that the response of C4 species to elevated CO₂ is not as clearcut as previously thought (Henderson *et al.* 1994), and that many C4 plants show significant photosynthetic and growth responses to CO₂. In a recent review, Poorter (1993) found an average growth enhancement of 22% for C4 species. Owensby *et al.* (1993) have also reported significant above-ground biomass increases in the C4 component of a tall-grass prairie site exposed to elevated CO₂. This was explained by the reduced water loss under high CO₂ of C4 species relative to competing C3 species, especially during a dry year. However, there also appears to be a primary direct enhancement of photosynthetic activity in elevated CO₂ in a number of C4 species (Sionit & Patterson 1984; Knapp *et al.* 1993), suggesting that the assumption of photosynthesis saturation at current CO₂ concentration may need to be re-evaluated.

Interacting environmental stresses can influence the response to elevated CO₂ in plants (Idso & Idso 1994; Curtis 1996; Lloyd & Farquhar 1996; Curtis & Wang 1998), and may do so differentially for different functional types. The literature of C4 responses to elevated CO₂ shows that environmental factors, especially those known to be of importance to C4 productivity and biogeographic distributions (high minimum temperatures and high light levels), may influence the relative CO₂-response. Responses of C4 species under stressful conditions may not emerge clearly from experiments employing growth conditions optimal for C3 plants. These factors could account for some of the poor responses to high CO₂ previously reported for environmentally controlled experiments, as opposed to significant responses meas-

ured more recently under natural field conditions (Owensby *et al.* 1993).

The purpose of this review is to assess critically from the literature, using meta-analytic methods (e.g. Curtis 1996; Curtis & Wang 1998), the physiological and growth responses of wild C4 grass species (family: Poaceae) to elevated atmospheric CO₂. To enable a critical test of current theories and perceptions, a similar literature review was carried out for the CO₂-responses of wild C3 grass species (Poaceae). This provides a clear comparison, controlled for genotypic and morphological variability. The influences of exposure and growth conditions were also analysed, in order to determine whether current understanding of the relative responses of C3 and C4 species, and resulting uncritical extrapolation to natural environments, may be biased by experimental conditions very different from natural conditions.

Materials and methods

Database compilation

The data analysed in this study were taken from published sources by investigators at the National Botanical Institute, South Africa, and the CO₂ Meta-Analysis Project, Ohio State University, USA. In cases where the two individual databases overlapped, data were used from the CO₂ Meta-Analysis Project only. Non-overlapping data were checked for consistency. The studies addressing C4 pathway grass species that were included in our analyses were as comprehensive as possible for all years (1980–97), while the studies addressing C3 pathway grass species were as comprehensive as possible for 1991–97, with most studies from 1980 to 1990 also included. The following criteria were used for incorporation of studies in the database:

- (a) The species was wild or semiwild, a member of the family Poaceae, and the photosynthetic pathway (C3 or C4) was either clearly stated or otherwise unambiguous.
- (b) Only data which included response means, sample sizes (N), and either standard deviation (SD) or standard error (SE) were used, since a weighted meta-analysis gives a more robust analysis than if resampling tests must be used to estimate variances or if an unweighted analysis is used (Rosenberg *et al.* 1997).
- (c) The paper was published between 1980 and 1997.
- (d) The ambient CO₂ treatment concentration was between 300 and 400 $\mu\text{mol mol}^{-1}$, and the elevated CO₂ treatment was between 550 and 750 $\mu\text{mol mol}^{-1}$.
- (e) Data were presented for individual plants, or for individual species where plants were grown in stands or in mixture with other species.
- (f) Only absolute data were used, not relative data such as relative growth rates.

(g) At least one of the following parameters was measured:

- A: Leaf-based light-saturated net CO₂ assimilation rates measured at the growth CO₂ concentration
- G_S: Leaf-based stomatal conductance measured at the growth CO₂ concentration
- WUE: Instantaneous leaf water use efficiency at the growth CO₂ concentration, either published as such or calculated from net CO₂ assimilation rates and transpiration rates
- TOTWT: Total plant biomass, either presented as such or calculated as the sum of above- and below-ground biomass.
- AGWT: Above-ground biomass
- BGWT: Below-ground biomass
- INDLA: Individual leaf area
- TILLERS: Number of tillers
- SLA: Specific leaf area either presented as SLA, or calculated as the inverse of specific leaf mass
- TNC: Concentration of total nonstructural carbohydrates in leaves, either presented as such or calculated as the sum of total sugar and starch concentrations, and expressed on a dry mass basis
- N: Leaf total nitrogen concentration expressed on a dry mass basis

The responses at elevated and ambient CO₂ were extracted either from tables, or manually digitized from figures. Where the interaction between CO₂ treatments and deliberately imposed light, temperature, water, or nutrient stress treatments was reported, the CO₂-response was entered separately under both levels of the stressful environmental factor. For those analyses testing a response to stress, all possible data in which plants were not stressed were included as controls for the meta-analysis ('no stress'), rather than only the data for nonstressed plants in studies reporting the response under intentional factorial stress treatments (controls within those studies). This necessitated careful decisions about how to code some treatment responses. For example, we determined that 'high nutrient levels' or normal nutrient levels (comparable to the field situation) were equivalent to 'no nutrient stress', and that 'high light levels' or light levels which were deemed normal or sufficiently high, were similarly equivalent to 'no light stress'. In these instances, we recorded 'none' for the level of stress. Furthermore, we utilized only the extreme levels of any given stress. That is, we included only 'low nutrients' (nutrient stress) and 'high nutrients' (no nutrient stress, or normal) in our analyses, and did not include intermediate levels (e.g. 'medium nutrient levels'). Studies which provided data on interactions with environmental stresses are identified in Appendix 3 (C3) and Appendix 4 (C4).

Where additional environmental stresses (such as salinity or ozone treatments) were imposed factorially, only the CO₂-response at the ambient, nonstressful level of this other factor was used. Unintentional stresses were not taken into account, except in the case of separately reported data for wet and dry years in some prairie studies. Where competition treatments were intentionally and differentially imposed, only the CO₂ response at the lowest level of competition was used.

Response parameters were combined whenever appropriate in order to overcome the problem of low sample sizes. For example, rather than differentiate between what some authors termed root biomass and others termed below-ground biomass, we pooled these data and report them as below-ground biomass. Thus, while we lost some potential detail in the analysis, we improved our ability to generalize and distinguish among effects (Gurevitch & Hedges 1993).

In order to test for potential influences of exposure methodology on the responses to elevated CO₂, the following categorical variables were assigned to each data entry:

- (i) Pot size: ≤ 10 L, > 10 L, or in-ground. These size classes have been previously used in a similar meta-analytic review (e.g. Curtis 1996).
- (ii) Duration of exposure (from treatment initiation until measurement): ≤ 60 days, 61–120 days, > 120 days. Where repeated measurements were taken, only the last measurement was used (usually at harvest). However, in some field studies showing marked seasonal responses, declining towards the end of the growth season, a single date at or just after the mid-season peak was chosen.
- (iii) Exposure method: GC=indoor controlled-environment growth chamber, GH=outdoor enclosed mini-greenhouse or enclosed portion of greenhouse, OTC=open-top chamber in the field or greenhouse, FACE=free-air CO₂ enrichment.

The database used for the meta-analysis comprised 62 papers (Appendix 1). Other papers on C4 grass responses to elevated CO₂ which did not meet the criteria for meta-analysis are given in Appendix 2 to provide a complete reference list.

Meta-analyses

Meta-analyses were conducted with MetaWin (Rosenberg *et al.* 1997), using the natural log of the response ratio (response in elevated CO₂/response in ambient CO₂) as our metric (Hedges *et al.* 1999). We used the mixed-effects model in our analyses, because of the large number of diverse studies examined and the assumption that there is random variation among studies in the effects in which we are interested. Consequently, the confidence intervals

generated are larger than those of a fixed-effects model, and as such represent potentially more conservative interpretation. In general, means of single response variables were considered significantly different from zero (significant response to elevated CO₂) if their 95% confidence intervals did not overlap zero. Similarly, means of two different response variables (e.g. stress treatment classes) were considered significantly different from each other if their 95% confidence intervals did not overlap. Some results are also discussed in terms of trends and tendencies in order to highlight interesting comparisons, even if they did not satisfy this statistical guideline. For a more detailed description of the statistical approach see Curtis & Wang (1998) and Hedges *et al.* (1999).

Results

Sample sizes for all variables presented in the Figures are given in Table 1.

Relative CO₂-responses of C3 and C4 species

CO₂ responses of the full data set, including responses under interacting stress variables, are presented as the mean percentage change in elevated CO₂ (Fig. 1a). Net CO₂ assimilation rates (*A*) increased significantly in both C3 and C4 species, by 33% and 25%, respectively. Stomatal conductances (*G_s*) decreased significantly by 24% and 29% for C3 and C4, respectively. Increases in instantaneous leaf water use efficiency (WUE) were significant only in C4 species (72%); the sample size for C3 was small and variability high. Total plant biomass (TOTWT) was enhanced in both C3 (44%) and C4 species (33%). C3 species showed greater CO₂-induced increases in above-ground biomass (AGWT, 38%) and below-ground biomass (BGWT, 44%), where these were reported individually, than C4 species. This suggests a deficiency in data for C4 biomass partitioning into above- and below-ground components, as the smaller effect here does not concur with the larger positive result for TOTWT. Due to reporting shortcomings, the data set for above- and below-ground biomass was often drawn from a different set of publications than that for total plant biomass, likely contributing to the lack of correspondence between the results for the three variables. Individual leaf area (INDLA) increased by 15% and 25% and tiller numbers increased by 27% and 14% in C3 and C4 species, respectively. C3 species showed greater decreases in specific leaf area (SLA, 19%) and foliar total nitrogen (N) concentrations (21%) than C4 species. Only C3 species showed significantly increased foliar total nonstructural carbohydrate (TNC) concentrations (37%) in elevated CO₂.

Table 1 Number of data entries (N) used for analyses of the CO₂-responses of C₃ and C₄ grasses. Abbreviations of variables as for Fig. 1. 'None' refers to no stress; 'low' or 'high' refers to stress. For pot size, category numbers represent (1) <10 L (2) >10 L (3) in-ground. For duration, category numbers represent (1) <60 days (2) 61-120 days (3) >120 days. For method, category numbers represent (1) GC (2) GH (3) OTC (4) FACE.

Variable	Photo pathway	CO ₂ -responses (Fig. 1)		Environmental stress (Fig. 2)			Exposure methods/Growth conditions(Figs 3-5)			
		All stresses	No stress	Light none;low	Temp. none; low/high	Water none;low	Nutrients none;low	Pot size cat.1;2;3	Duration cat. 1;2;3	Method cat. 1;2;3;4
A	C ₄	48	15		38 ; 8	42 ; 6		34 ; - ; 12	29 ; 7 ; 12	29 ; 6 ; 13 ; -
	C ₃	68	8				41 ; 19	27 ; 32 ; 9	55 ; 8 ; 5	50 ; 11 ; 3 ; 4
G _s	C ₄	47	16		40 ; 5	41 ; 6	44 ; 3	35 ; - ; 10	28 ; 7 ; 12	27 ; 7 ; 13 ; -
	C ₃	7	3					4 ; - ; -	4 ; - ; 3	4 ; - ; 3 ; -
WUE	C ₄	13	7							
	C ₃	2	2							
TOTWT	C ₄	25	6	22 ; 3				20 ; - ; 2	18 ; 2 ; 5	
	C ₃	71	9				54 ; 13	49 ; 17 ; 5	49 ; 13 ; 9	
AGWT	C ₄	19	10			16 ; 3		19 ; - ; -	19 ; - ; -	18 ; - ; - ; -
	C ₃	40	23		36 ; 3		37 ; 3	18 ; 13 ; 9	7 ; 15 ; 18	11 ; 10 ; 17 ; 2
BGWT	C ₄	9	4					9 ; - ; -		
	C ₃	30	8				21 ; 9	19 ; 10 ; -		
INDLA	C ₄	14	5			10 ; 4		9 ; - ; 2	9 ; - ; 5	10 ; 2 ; 2 ; -
	C ₃	8	0					6 ; - ; -	2 ; 2 ; 4	7 ; - ; - ; -
TILLERS	C ₄	9	5						4 ; - ; 5	5 ; 2 ; 2 ; -
	C ₃	12	6						2 ; 7 ; 3	8 ; - ; 4 ; -
SLA	C ₄	19	6							
	C ₃	21	5							
TNC	C ₄	4	0							
	C ₃	12	11							
N	C ₄	15	5							
	C ₃	37	10				22 ; 11			

Influence of environmental stress

Under nonstressful growth conditions (Fig. 1b), the relative mean stimulation of photosynthetic rate in C₃ species increased from 33% to 53%, and below-ground biomass enhancement rose from 44% to 57%. However, both changes were not significant according to the 95% confidence interval (CI) overlap test. The CO₂-responses of all other variables remained similar compared to the 'all stresses' analysis (Fig. 1a). By contrast, when interacting stresses were removed from the database for C₄ species, the response of total biomass to elevated CO₂ decreased from 33% to 26%, the tillering response was reduced (from a 14% increase to a 10% increase), but stimulation of individual leaf area rose from 25% to 30%. Again, these responses were not significant according to the CI overlap test.

The influence of environmental stresses was further explored by comparing the CO₂-responses of deliberately stressed plants with the responses of all other plants (not deliberately stressed) for each stress variable individually (Fig. 2). Only results which can be interpreted with

reasonable confidence, taking into account the sample size (>2), the confidence interval, and the power to draw robust statistical conclusions, are presented. For example, no studies addressing the interactions between elevated CO₂ and light or water stress in C₃ grass species existed or were suitable for use in the meta-analysis, and in many other cases the number of studies for a particular measurement category and stress factor were too small, or did not exist.

In C₄ species (Fig. 2a), low light, low temperature, or low nutrient supply levels did not alter the mean responses to elevated CO₂. Droughting treatments (low water supply) similarly did not alter the CO₂-responses of gas exchange in C₄ species, but resulted in the loss of a significant CO₂-response for above-ground biomass and individual leaf area (95% confidence intervals overlap zero), compared to plants that were not water stressed. There were no data for C₄ plants under high temperature stress, probably because high temperatures are not regarded as being potentially harmful to C₄ plants as they are for C₃ plants. In C₃ species (Fig. 2b), on the other hand, abnormally high temperatures increased the mean

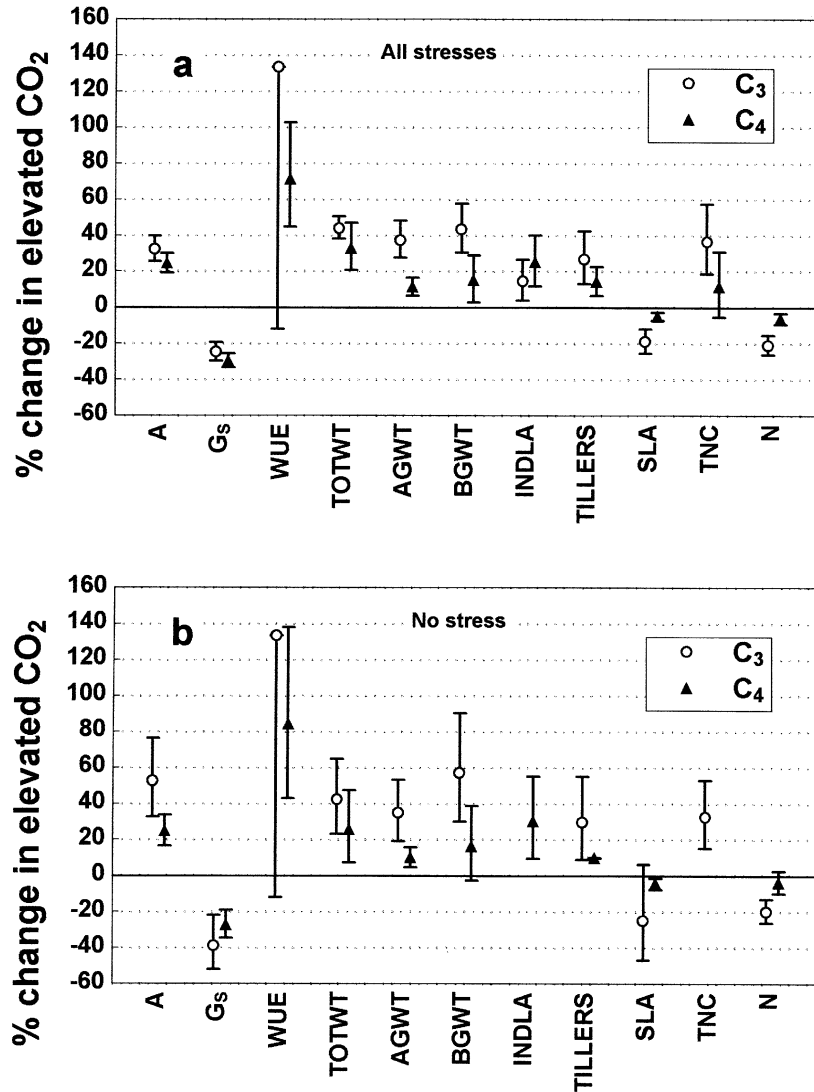


Fig. 1 Comparative photosynthetic, growth, morphological, and chemical responses of wild C3 and C4 grass species to elevated atmospheric CO₂ concentrations. (a) full data set including responses under all levels of environmental interactions other than CO₂. (b) selected data set of CO₂-responses under nonstressful environmental conditions. Abbreviations: A, net CO₂ assimilation rate; G_s, stomatal conductance; WUE, instantaneous leaf water use efficiency; TOTWT, total plant dry weight; AGWT, above-ground dry weight; BGWT, below-ground dry weight; INDLA, individual leaf area; TILLERS, tiller number; SLA, specific leaf area; TNC, leaf total nonstructural carbohydrate concentration; N, leaf total nitrogen concentration. No data were available for INDLA (C3 species) and TNC (C4 species) for nonstressful environmental conditions. Data represent percentage change in elevated CO₂ with 95% confidence intervals.

above-ground biomass response to elevated CO₂, but this tendency was not significant. Low nutrient stress did not alter the mean CO₂-response of photosynthesis and leaf nitrogen concentration in C3 species. By contrast, mean CO₂-induced increases in total plant, above- and below-ground biomass tended to diminish under low nutrient supply levels, although these changes were not significant.

Exposure methods and growth conditions

The effects of exposure methods and growth conditions on the relative responses to elevated CO₂ are presented in Figs 3, 4, 5. Those variables for which data for at least two categories were available for either the C3 or C4 data set, and which had reasonable sample sizes and the potential for meaningful statistical inferences, are presented. These are matched with

the results for the corresponding variable in the other (C3 or C4) data set, even if the sample sizes are small and categories missing. This was done in order to allow at least a rudimentary comparison between C3 and C4 species. Even though this comparison is largely fragmentary, it exposes gaps in the knowledge base, particularly with regard to the lack of information from long-term studies in the field, and could provide a guideline for future studies (and publication of existing data).

An increasing volume of available rooting space ('pot size', Fig. 3) allowed for slightly greater CO₂-induced increases in photosynthetic rate in C3 species, although not significantly according to the CI overlap test. The mean photosynthetic CO₂-response in C4 species was not altered. Stomatal conductances were reduced more strongly in C4 species growing in open ground than in small pots. Large positive responses in

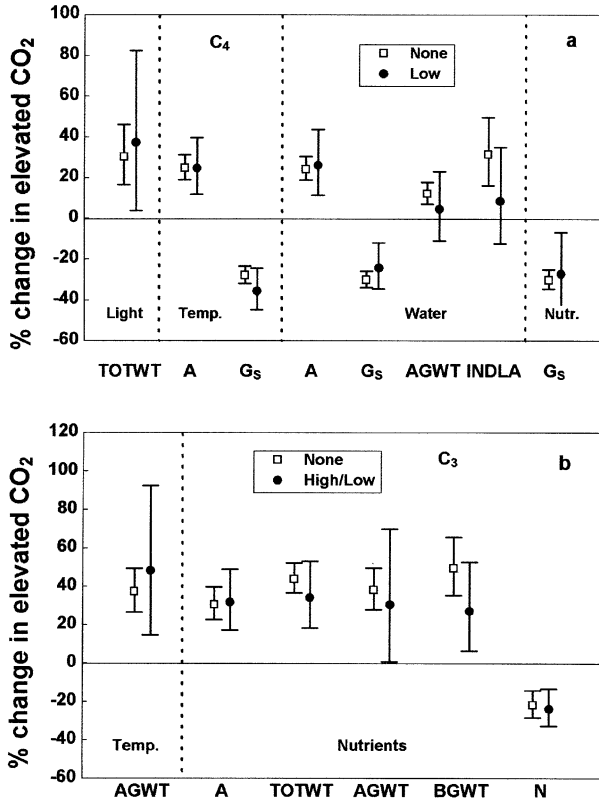


Fig. 2 CO₂-responses of wild C4 (a) and C3 (b) grass species as influenced by interaction with low light, low or high temperature, low water, or low nutrient stress. Abbreviations as for Fig. 1. Data represent percentage change in elevated CO₂ with 95% confidence intervals.

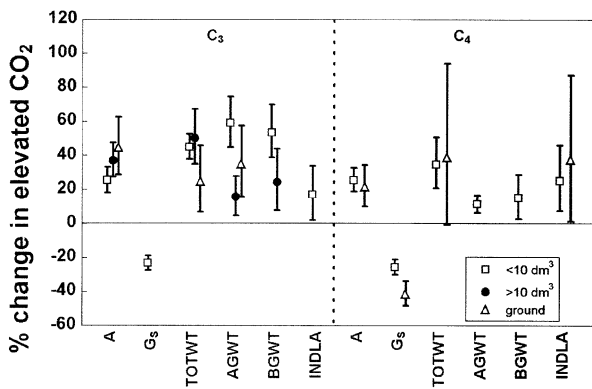


Fig. 3 Effect of pot size on CO₂-response of C3 and C4 grass species. Legend refers to pot size in dm³, or plants grown in-ground. Abbreviations as for Fig. 1. Data represent percentage change in elevated CO₂ with 95% confidence intervals.

biomass in C3 species were favoured by growth in smaller rooting volumes, as supported by no or only minimal overlap in the confidence intervals between pot size classes. This effect on biomass was not

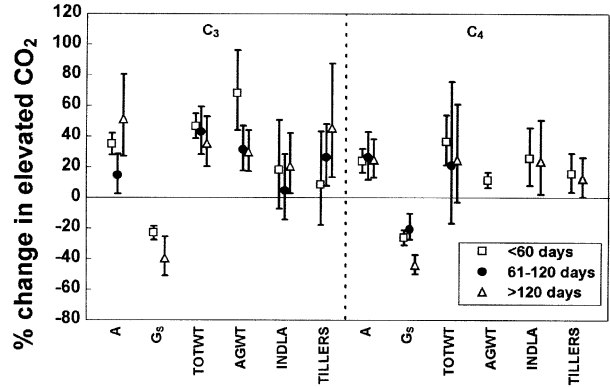


Fig. 4 Effect of duration of exposure on the CO₂-response of C3 and C4 grass species. Abbreviations as for Fig. 1. Data represent percentage change in elevated CO₂ with 95% confidence intervals.

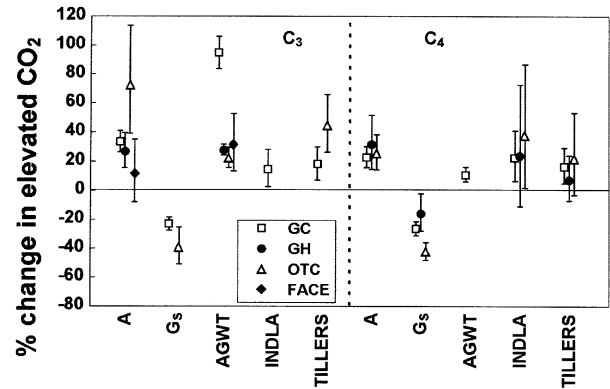


Fig. 5 Effect of exposure method on the CO₂-response of C3 and C4 grass species. Abbreviations as for Fig. 1. In the legend, GC, growth chamber; GH, greenhouse; OTC, open-top chamber; FACE, free-air CO₂ enrichment. Data represent percentage change in elevated CO₂ with 95% confidence intervals. Note that GC, GH and OTC treatments typically have 2× ambient [CO₂] (650–700 μmol mol⁻¹), and that FACE is typically about 550 μmol mol⁻¹ [CO₂].

discernible in C4 species, although interpretation here is strongly limited by insufficient sample sizes. Significant increases in individual leaf area in C4 species in high CO₂ were measured in plants growing both in small pots and in the ground.

The mean CO₂-responses for photosynthetic rate and conductance in C3 species were greater in the longer term (> 120 days, Fig. 4) than in the medium term (61–120 days). The mean above-ground biomass response, by contrast, was greatest in the short term (<60 days). The positive tillering response to elevated CO₂ achieved significance (95% confidence interval not overlapping zero) only after 60 days of exposure to high CO₂, and continued to

increase with experimental duration, although not significantly. In C4 species, relative decreases in conductance were significantly greater in the longer term (> 120 days). The biomass response to elevated CO₂ appeared to decrease somewhat after 60 days (although small sample sizes and large confidence limits preclude a clear interpretation), so that mean biomass increases were no longer significant after 60 days (confidence intervals overlap zero). Similar increases in leaf area in elevated CO₂ were found both in the short and longer term.

Increases in photosynthetic rates in elevated CO₂ appeared to be greatest when C3 species were grown in open-top chambers (73%), and smallest in FACE systems (12%, Fig. 5), although the latter is probably attributable to the generally lower CO₂ concentrations used in these systems than in the other types of growth facilities. In both C3 and C4 species, mean decreases in conductances were greatest in OTCs. Above-ground biomass increases were found for all exposure methods in C3 species, but responses were particularly high in growth chambers (95% increase in GC compared to 23–32% increases in other facilities). The mean tillering response was greater in OTCs (45% increase) than in growth chambers (18%). In C4 species, CO₂-induced increases in photosynthetic rates were similar in all exposure facilities. Leaf area increases were found for most exposure methods (with the possible exception of greenhouses, where confidence intervals overlapped zero), and the tillering response to elevated CO₂ was small for all methods and not significant in GHs and OTCs (CI overlapped zero), but in both cases interpretation was hampered by insufficient sample sizes.

Discussion

Relative CO₂-responses of C3 and C4 species

The results of this meta-analysis confirm the widely held view that the relative responses of C4 species to elevated CO₂ are usually smaller than those for C3 species, especially for growth under nonstressful environmental conditions. Nevertheless, differences in CO₂ response between C3 and C4 grass species are not as large as current perceptions have it. A similar conclusion was drawn from a previous semiquantitative minireview of C3 vs. C4 responses (Poorter 1993). The present analysis shows that C4 grasses are certainly responsive to elevated CO₂ particularly with regard to gas exchange and leaf area development.

Photosynthetic stimulation of C4 species is, surprisingly, comparable to that of C3 species. This contradicts the general view that C4 photosynthesis does not increase in elevated CO₂, due to the specialized CO₂ concentrating mechanism in C4 leaves (Bowes 1993).

Many of the definitive early studies of C4 photosynthesis were performed on crop species, such as maize, which appears to be CO₂-saturated at ambient CO₂ levels and shows very low responsiveness to higher CO₂ concentrations, compared to wild C4 species (Ziska & Bunce 1997). A closer examination of gas exchange in other C4 grasses reveals that photosynthesis is not necessarily saturated at current CO₂ levels and can increase at higher c_i (Sionit & Patterson 1984; Imai & Okamoto-Sato 1991). This simple explanation may account for the majority of cases of photosynthetic stimulation in C4 species. For example, LeCain & Morgan (1998) showed that photosynthesis was not saturated at ambient CO₂ concentration in any of the six wild C4 grass species studied. Ziska & Bunce (1997) similarly measured higher photosynthetic rates at elevated CO₂ in 8 out of 10 C4 species studied, due to the effect of increased c_i . Another study has shown that under favourable growth conditions (high light), the operational c_i of a tropical C4 grass was close to the inflection point of the A/c_i response, so that increasing CO₂ supply caused slight enhancements in the CO₂ assimilation rates, and improved growth (Ghannoum *et al.* 1997). On the other hand, under growth-limiting conditions (low light), the operational c_i was well above the CO₂ saturation level for photosynthesis, and no photosynthetic or growth response to elevated CO₂ was measured. The relative 'saturation level' may therefore change with changing conditions, and this may play a role in photosynthetic responses to elevated CO₂ in wild C4 grasses.

Stimulation of photosynthetic rates due to increases in c_i represents a simple short-term effect. In addition, longer term biochemical changes, such as altered enzyme efficiencies, or altered regeneration rates of phosphoenolpyruvate (PEP) or ribulose-1,5-bisphosphate (RuBP), may develop in elevated CO₂. These are termed 'regulatory' or 'acclimatory' responses and change the shape of the A/c_i response. Unlike for many C3 species (Wullschlegel 1993), instances of up- or downregulation of photosynthetic capacity in C4 species have not been given much attention in the literature as they have appeared to be rare. Sage (1994) concluded that little adjustment is found in the A/c_i response in C4 species under elevated CO₂ (see also Ziska & Bunce 1997), except possibly downregulation under conditions of nutrient deficiency (Wong 1979; Morgan *et al.* 1994; Ghannoum & Conroy 1998). Nevertheless, photosynthetic downregulation has also been measured under conditions not apparently stressful (Read *et al.* 1997; LeCain & Morgan 1998). Many earlier reports of unchanged or reduced photosynthetic rates in C4 species in elevated CO₂ may have been due to the acclimation phenomenon, but are not identifiable without full A/c_i measurements (e.g. Wray & Strain 1986). The mechanisms of photosynthetic

acclimation in C4 species are apparently not related to feedback inhibition resulting from carbohydrate (TNC) accumulation, or to reductions in leaf nitrogen (N) concentrations (Read *et al.* 1997; LeCain & Morgan 1998), as they are in C3 species (Stitt 1991; Sage 1994; Cotrufo *et al.* 1998). The meta-analysis confirmed that accumulation of TNC and reductions in leaf N in elevated CO₂ are insignificantly low in C4 species. There is currently no available information on the possible mechanisms of photosynthetic acclimation in C4 species, and this warrants further attention.

Natural seasonal dynamics of photosynthetic capacity in C4 species may also influence the response to elevated CO₂. In young *Themeda triandra* (red grass) plants with high assimilation rates, photosynthetic upregulation resulted in increased photosynthetic rates in high CO₂ (Ludwig 1996), but downregulation was measured in the same set of plants later in the season (when absolute rates were reduced), which led to similar or reduced photosynthetic rates at high compared to ambient CO₂ (Wand, unpublished data). Growth enhancement, particularly of leaf area, was linked to this early response. Detailed measurements of A/c_i responses in C4 and C3 grass species growing in elevated CO₂ in a field experiment in South Africa (utilization of a natural CO₂ spring) have also shown photosynthetic upregulation in *Themeda triandra* during the active growth season (Wand, unpublished data). Similarly, significant increases in CO₂ assimilation rates in the salt marsh C4 species *Spartina patens* in high CO₂ were confined to the early season, when absolute rates of assimilation were highest (Ziska *et al.* 1990). Knapp *et al.* (1993) have also reported upregulation in *Andropogon gerardii* (big bluestem), and Chen *et al.* (1994) modelled this response. Recent reports indicate that C4 photosynthetic physiology may change with progressive developmental stages, showing more similarities with C3 physiology (lower CO₂-concentrating ability) when leaves are young or senescent, compared to mature leaves (Dai *et al.* 1995; He & Edwards 1996). This was tentatively proposed as an explanation for ontogenetic shifts in CO₂-responsiveness (Ghannoum *et al.* 1997), but subsequent work on C4 grass species does not support this explanation, as C4 photosynthetic characteristics were already fully developed in young leaves (Ghannoum *et al.*, 1998). In conclusion, this aspect of C4 response to elevated CO₂ requires more attention, as conflicting evidence also exists. For example, photosynthetic downregulation was found in both young and older *Bouteloua gracilis* (blue grama) plants (Read *et al.* 1997). Furthermore, seasonal dependencies of C4 growth responses, in contrast with responses of carbon assimilation, are not evident in many field-based elevated CO₂ experiments (Curtis *et al.* 1989; Kirkham *et al.* 1991; Hamerlynck *et al.* 1997).

The issue of whether biochemical differences between C4 photosynthetic subtypes may shed light on the reasons for interspecific differences in CO₂ responsiveness (Henderson *et al.* 1994) is beyond the scope of the present review, but we make some brief comments. The three C4 subtypes (NADP-ME (NADP-malic enzyme), PCK (phosphoenolpyruvate carboxykinase), and NAD-ME (NAD-malic enzyme)) exhibit increasing levels of 'leakiness' to CO₂ from the bundle sheath to the mesophyll, in the above order (Hattersley 1982; Furbank & Hatch 1987; Jenkins *et al.* 1989; Brown & Byrd 1993; but see Hatch *et al.* 1995). This amounts to a loss of between 10 and 40% of carbon fixed by PEP carboxylase, which could, conceivably, be counteracted by increased CO₂ supply from the atmosphere. Recent studies investigating the relative responsiveness of the subtypes to elevated CO₂ yielded counter-intuitive results, with the least 'leaky' NADP-ME showing the largest responses (LeCain & Morgan 1998; Wand, unpublished data). Nevertheless, this line of investigation may well contribute to an improved understanding of photosynthetic responses of C4 plants to elevated CO₂.

Elevated CO₂ has significant positive effects on plant water relations in both C3 and C4 grass species, via reductions in stomatal conductance (G_s). In fact, this response, coupled with reduced transpirational water loss and the corresponding increases in WUE, are probably the most ubiquitous responses to elevated CO₂ for almost all plant functional types (Gifford & Morison 1985; Chaves & Pereira 1992; Tyree & Alexander 1993). C4 and C3 responses in G_s to elevated CO₂ were similar (Fig. 1a), but clear interpretation is hampered by the fact that fewer data are available for C3 Poaceae. The decrease in G_s in C4 species is consistent across a range of environmental stresses, but greatest in plants grown in the ground and exposed to elevated CO₂ for more than 120 days. This suggests that developmental changes in G_s , possibly related to altered stomatal sizes or densities, may occur as leaves mature in high CO₂. Information on long-term changes in G_s (e.g. changing stomatal densities) is limited (Ghannoum *et al.* 1997). It is generally thought that CO₂-induced reductions in G_s are primarily short-term effects, but some researchers have measured acclimatory responses in stomatal physiology in C4 species, as shown by the responses of conductance to increasing CO₂ (G_s/c_i curves) (Morgan *et al.* 1994; Read *et al.* 1997; LeCain & Morgan 1998). This can take the form of either upregulation (higher G_s at equivalent c_i for leaves grown in elevated CO₂), or downregulation (reduced G_s at low c_i in elevated CO₂). Studies in the greenhouse and field (Wand, unpubl. data) showed that G_s was significantly reduced under increasing CO₂ levels in the short term (changes in the cuvette CO₂ concentration) in all seven C4 grass species studied. In addition, either upregulation or

downregulation of G_s was also found in the longer term (a treatment effect) in some species, and this developmental response appeared to depend on season or environmental conditions (e.g. water stress). Reduced transpirational water loss in elevated CO_2 and the resulting improvement in soil water content over the course of the growing season, as reported for the tall-grass prairie (Kirkham *et al.* 1993), are likely to be reflected in longer term changes in stomatal conductances.

The stimulation of C4 whole plant growth under elevated CO_2 (mean of 33%, 95% confidence interval 21%–47%) is slightly higher than the 22% reported by Poorter (1993). Growth stimulation could be either a direct effect of greater carbon assimilation rates (discussed above), or an indirect effect of improved soil and leaf water relations resulting from reduced stomatal conductances and transpirational water loss (Knapp *et al.* 1993; Owensby *et al.* 1993). Cell elongation and blade extension rates in developing grass leaves are positively correlated with leaf water potentials (Boyer 1970; Toft *et al.* 1987). Although not included in the meta-analysis, the C4 literature database clearly showed a consistent and significant positive increase in shoot water potentials in grasses exposed to elevated CO_2 (e.g. Kirkham *et al.* 1993; Hamerlynck *et al.* 1997).

The growth response of C3 species in this review is a little larger than that for C4 species (44%) and comparable to the C3 herbaceous monocot component of Poorter's database (42%). We tentatively support Poorter's conclusion that differences in growth stimulation between C3 and C4 plants are probably not as large as suggested by current perceptions. Unfortunately, the responses for above- and below-ground biomass in C4 species in this meta-analysis do not match those for whole-plant biomass, and care must be taken in interpretation. Also, many field studies using C4 species have not reported biomass responses adequately, probably due to logistical difficulties and an unwillingness to disturb the ecosystem in longer term experiments. We need more information on whether increased carbon assimilation rates will lead to sustained enhanced biomass production in C4-grass-dominated ecosystems such as prairies and savannas, which comprise a large percentage of productive land surface (Hall *et al.* 1995). This would help to improve our models of global carbon dynamics. Currently, the potential of C4-grass-dominated ecosystems as significant carbon sinks is considered small, but this may need to be re-assessed.

An interesting contrast emerged regarding the morphological development of C3 and C4 species under elevated CO_2 . C3 species generally develop more tillers, with only small increases in leaf area, but decreased specific leaf areas (increased leaf density or thickness). C4 species, on the other hand, appeared to respond mainly

with increased leaf areas, and smaller increases in tiller numbers. This contrast may indicate a greater sensitivity in C4 species to self-shading of the basal nodes from which tillers are initiated (Deregibus *et al.* 1985; Everson *et al.* 1988), and may provide the mechanism for growth stimulation even under moderate photosynthetic enhancement. Gradually increasing canopy leaf areas, leading to a progressive increase in whole-canopy carbon assimilation rates, would result in a continuously greater supply of carbon products to support enhanced growth. Early increases in leaf area, leaf area duration (the cumulative leaf area over the growth period), leaf area ratio (the proportion of leaf area to plant biomass), plant height and total plant biomass of C4 species in elevated CO_2 have been reported by Patterson & Flint (1980), Riechers & Strain (1988) and Ackerly *et al.* (1992). Early responses in biomass and leaf area, which persist for the whole growth period, have also been found for some C3 species (Bowler & Press 1993), but the stimulation of leaf area, in particular, appears to be characteristic of the CO_2 -response of C4 species. Coleman & Bazzaz (1992) and Ackerly *et al.* (1992) came to the conclusion that standing photosynthetically active leaf area (net leaf area production and loss) in a C4 species was the primary influence on growth responses in elevated CO_2 .

Influence of environmental stress and growth methodology

Environmental stresses tend to reduce (although not significantly) the potential CO_2 -response in C3 species, as evidenced by the suppression of mean photosynthetic and below-ground biomass responses when all stresses are included in the analysis (Fig. 1a,b), as well as the reductions in mean growth responses when nutrients are limiting (Fig. 2b). By contrast, C4 species were generally not negatively impacted by environmental stresses, although leaf area stimulation was sensitive to water stress (Fig. 2a). The current view that CO_2 -responses in C4 graminoids are particularly marked under conditions of water stress (Nie *et al.* 1992; Knapp *et al.* 1993; Owensby *et al.* 1993; Ham *et al.* 1995) was not borne out by the meta-analysis, possibly due to the lack of data suitable for inclusion in the database. Many of these studies do not present plant- and leaf-level responses, concentrating on canopy-level gas fluxes instead.

C4 graminoids may well benefit from increased CO_2 supply under some stressful environmental conditions, such as low soil fertility. However, there is a lack of information about the changes in plant nitrogen use efficiency in C3 and C4 graminoids in elevated CO_2 . This understanding may be critical in predicting changes in their relative competitive abilities, as it has been suggested that competitive advantage,

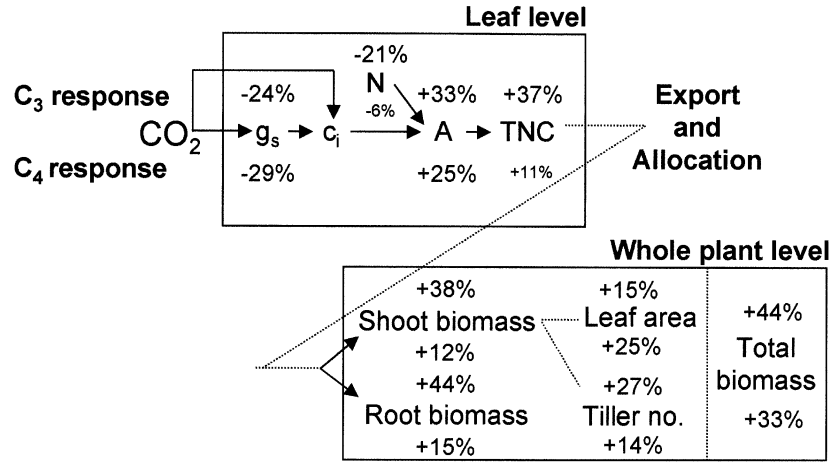


Fig. 6 Summary scheme of the CO₂-response levels (% change) and their relationships under all conditions (full data set). C₃ responses are given above and C₄ responses given below the stated parameter. Response levels printed in small font are not significantly different from zero. Abbreviations as for Fig. 1, and c_i=intercellular CO₂ concentration.

especially in grasslands, may be due to a greater ability to extract nitrogen from the soil (Tilman 1990). This in turn may be associated with greater plant and photosynthetic nitrogen use efficiency (Richardson *et al.* in press).

Contrary to strong evidence for nongraminoid C₃ species showing reduced CO₂-responsiveness in small rooting volumes (Arp 1991; McConnaughay *et al.* 1993, 1996), C₃ grass species were more responsive in small pots, with respect to above- and below-ground growth. In C₄ species, rooting volume did not appear to play a role in the biomass response to CO₂, but more data are needed to confirm this as the sample size was too small for a confident interpretation. Nevertheless, field studies with C₃ species have not supported the expected significant productivity increases based on earlier pot studies, and predictions of competitive advantages over C₄ species should be cautiously reviewed. In addition, above-ground biomass enhancement of C₃ species diminished with increasing duration of exposure to elevated CO₂, even though tiller number appeared to be stimulated, which suggests that individual tiller size is progressively reduced in this group.

In conclusion, responses to elevated CO₂ in wild C₄ and C₃ Poaceae at the leaf and whole plant levels are summarised in Fig. 6, and demonstrate many trends common to both photosynthetic types. At the leaf level, the greater carbohydrate accumulation and greater reductions in leaf nitrogen concentration in the C₃ type alone differentiated the types, and constituted the only evidence for so-called 'sink limitation' which is often invoked in elevated CO₂ studies on C₃ dicots. Average photosynthetic responsiveness did not concur with predictions based solely on photosynthetic theory. However, at the shoot level, there were clearcut differences between types resulting from disparate effects on above-

ground morphologies. These, rather than photosynthetic differences between the types, might be of greater importance when evaluating responses to elevated CO₂.

The combined shoot structural and water use efficiency responses of these functional types are likely to have consequential implications for the water balance of important catchments and rangelands throughout the world, especially in semiarid subtropical and temperate regions. Improved water relations would be highly beneficial to C₄ grasses growing in marginal semiarid sites where growing season may be limited by soil water availability, such as over much of southern Africa and parts of North America. The results of this meta-analysis suggest that it may be premature to predict that the C₄ type will lose its competitive advantage in certain regions as CO₂ levels rise, based solely on differential photosynthetic mechanisms (Collatz *et al.* 1998).

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Appendix 1

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Appendix 2

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Appendix 3

References, C3 species, exposure and growth conditions, and interaction with other environmental stresses, for studies used in the analysis. GC, growth chamber; GH, greenhouse; OTC, open-top chamber; FACE, free-air CO₂ enrichment

Reference	C3 species	Exposure facility	Pot size (L)	Duration (days)	Interacting stresses		
					Nutr.	Temp.	Water
Baxter <i>et al.</i> (1994a)	<i>Agrostis capillaris</i>	OTC	0.7	79			
	<i>Festuca vivipara</i>	OTC	0.7	189			
	<i>Poa alpina</i>	OTC	0.7	105			
Baxter <i>et al.</i> (1994b)	<i>Agrostis capillaris</i>	OTC	0.4	43			
	<i>Festuca vivipara</i>	OTC	0.4	189			
	<i>Poa alpina</i>	OTC	0.4	105			
Baxter <i>et al.</i> (1995)	<i>Agrostis capillaris</i>	OTC	0.7	79			
	<i>Festuca vivipara</i>	OTC	0.7	189			
	<i>Poa alpina</i>	OTC	0.7	105			
Baxter <i>et al.</i> (1997)	<i>Poa alpina</i>	GC	2.5	50			
Bowler & Press (1993)	<i>Agrostis capillaris</i>	GC	3.8	58			*
	<i>Nardus stricta</i>	GC	3.8	63			*
Bowler & Press (1996)	<i>Agrostis capillaris</i>	GC	25	42			*
	<i>Nardus stricta</i>	GC	25	49			*
Campbell <i>et al.</i> (1995)	<i>Agrostis capillaris</i>	GC	0.8	28			*
	<i>Bromus willdenowii</i>	GC	0.8	42			*
	<i>Dactylis glomerata</i>	GC	0.8	28			*
	<i>Festuca arundinacea</i>	GC	0.8	42			*
	<i>Lolium multiflorum</i>	GC	0.8	28			*
	<i>Lolium perenne</i>	GC	0.8	42			*
	<i>Phalaris aquatica</i>	GC	0.8	28			*
Casella <i>et al.</i> (1996)	<i>Lolium perenne</i>	GH	220	720			*
Ferris <i>et al.</i> (1996)	<i>Lolium perenne</i>	GH	3.7	133			*
Fischer <i>et al.</i> (1997)	<i>Lolium perenne</i>	FACE	G	426			*
Fitter <i>et al.</i> (1996)	<i>Festuca ovina</i>	OTC	G	730			
Ghannoum <i>et al.</i> (1997)	<i>Panicum laxum</i>	GH	7.0	49			
Gloser & Bartak (1994)	<i>Calamagrostis epigejos</i>	GC	0.5	21			
Greer <i>et al.</i> (1995)	<i>Lolium perenne</i>	GC	1.2	28/56			*
	<i>Agrostis capillaris</i>	GC	1.2	28/56			*
Hakala & Mela (1996)	<i>Festuca pratensis</i>	OTC	G	510			*
Jackson & Reynolds (1996)	<i>Avena fatua</i>	OTC	30	135			*
	<i>Bromus hordeaceus</i>	OTC	30	135			*
	<i>Lolium multiflorum</i>	OTC	30	135			*
	<i>Vulpia microstachys</i>	OTC	30	135			*
	<i>Avena barbata</i>	OTC	G	430			
Jackson <i>et al.</i> (1995)	<i>Avena barbata</i>	OTC	G	790			
	<i>Avena sativa</i>	GH	3.1	30			
Jones <i>et al.</i> (1996)	<i>Lolium perenne</i>	OTC	G	735			
Larigauderie <i>et al.</i> (1988)	<i>Bromus mollis</i>	GC	3	129			*
Leadley & Stöcklin (1996)	<i>Bromus erectus</i>	GC	24.3	126			
	<i>Festuca ovina</i>	GC	24.3	126			
Lenssen <i>et al.</i> (1995)	<i>Puccinellia maritima</i>	GH	1.8	28			
Marks & Clay (1990)	<i>Lolium perenne</i>	GC	0.5	70			*
Morgan <i>et al.</i> (1994a)	<i>Pascopyrum smithii</i>	GC	20.4	460			*
Newton <i>et al.</i> (1995)	<i>Lolium perenne</i>	GC	150	340			
Nie <i>et al.</i> (1992)	<i>Poa pratensis</i>	GH	G	61			*
Nijs <i>et al.</i> (1996)	<i>Lolium perenne</i>	FACE	G	23			*
Nijs <i>et al.</i> (1997)	<i>Lolium perenne</i>	FACE	G	143			*
Read <i>et al.</i> (1997)	<i>Pascopyrum smithii</i>	GC	6	49			*
Ryle <i>et al.</i> (1992)	<i>Lolium perenne</i>	GC	2	49			
Saebo & Mortensen (1995)	<i>Lolium perenne</i>	OTC	48	147			
	<i>Phleum pratense</i>	OTC	48	147			
Saebo & Mortensen (1996)	<i>Agrostis capillaris</i>	OTC	48	60			
	<i>Dactylis glomerata</i>	OTC	48	64			

Reference	C3 species	Exposure facility	Pot size (L)	Duration (days)	Interacting stresses		
					Nutr.	Temp.	Water
	<i>Festuca arundinaceae</i>	OTC	48	64			
	<i>Festuca duruiscula</i>	OTC	48	72			
	<i>Festuca pratensis</i>	OTC	48	72			
	<i>Festuca rubra</i>	OTC	48	71			
	<i>Poa pratensis</i>	OTC	48	72			
Schäppi & Körner (1996)	<i>Poa alpina</i>	OTC	G	310			*
Stewart & Potvin (1996)	<i>Poa pratensis</i>	GC	27.4	61			
	<i>Poa pratensis</i>	OTC	G	61			
	<i>Elymus athericus</i>	GH	1.8	65			
Stirling <i>et al.</i> (1997)	<i>Poa alpina</i>	GH	4.7	75			*
	<i>Poa annua</i>	GH	4.7	75			*
Teughels <i>et al.</i> (1995)	<i>Lolium perenne</i>	GH	6.2	60			
	<i>Festuca arundinaceae</i>	GH	6.2	30			
van de Staaij <i>et al.</i> (1993)	<i>Elymus athericus</i>	GH	1.8	65			
Volin & Reich (1996)	<i>Agropyron smithii</i>	GC	2.5	58			*
Wilsey (1996)	<i>Stipa occidentalis</i>	GC	2	86			*
Wilsey <i>et al.</i> (1997)	<i>Agropyron caninum</i>	GC	2.0	75			
	<i>Festuca idahoensis</i>	GC	2.0	75			
	<i>Briza subaristata</i>	GC	2.0	75			
	<i>Stipa occidentalis</i>	GC	2.0	75			
Zanetti <i>et al.</i> (1997)	<i>Lolium perenne</i>	FACE	G	913			
Ziska <i>et al.</i> (1991)	<i>Pharus latifolius</i>	OTC	12.5	100			

Appendix 4

References, C₄ species, exposure and growth conditions, and interaction with other environmental stresses, for studies used in the analysis. GC, growth chamber; GH, greenhouse; OTC, open-top chamber

Reference	C4 species	Exposure facility	Pot size (L)	Duration (days)	Interacting stresses		
					Temp.	Water	Light
Bowman & Strain (1987)	<i>Andropogon glomeratus</i>	GC	1	56			
Campbell <i>et al.</i> (1995)	<i>Digitaria sanguinalis</i>	GC	0.8	42	*		
	<i>Paspalum dilatatum</i>	GC	0.8	42	*		
Carlson & Bazzaz (1982)	<i>Setaria faberii</i>	GH	1	32			
	<i>Setaria lutescens</i>	GH	1	32			
Curtis <i>et al.</i> (1989)	<i>Spartina patens</i>	OTC	G	124			
Curtis <i>et al.</i> (1990)	<i>Spartina patens</i>	OTC	G	580			
Garbutt <i>et al.</i> (1990)	<i>Setaria faberii</i>	GH	1	78			
Ghannoum <i>et al.</i> (1997)	<i>Panicum antidotale</i>	GH	7	49			*
Gifford & Morison (1985)	<i>Paspalum plicatulum</i>	GC	3.2	131			*
Hamerlynck <i>et al.</i> (1997)	<i>Andropogon gerardii</i>	OTC	G	1255			*
Kirkham <i>et al.</i> (1991)	<i>Andropogon gerardii</i>	OTC	G	214			*
Knapp <i>et al.</i> (1993)	<i>Andropogon gerardii</i>	OTC	G	480			*
Marks & Clay (1990)	<i>Tridens flavus</i>	GC	0.5	70			
Marks & Strain (1989)	<i>Andropogon virginicus</i>	GC	0.5	16			*
Morgan <i>et al.</i> (1994a)	<i>Bouteloua gracilis</i>	GC	8	76			*
Morgan <i>et al.</i> (1994b)	<i>Bouteloua gracilis</i>	GC	20	190			
Newton <i>et al.</i> (1995)	<i>Paspalum dilatatum</i>	GC	150	340			
Nie <i>et al.</i> (1992)	<i>Andropogon gerardii</i>	GH	G	61			*
Polley <i>et al.</i> (1996)	<i>Schizachyrium scoparium</i>	GH	30	480			
Potvin & Strain (1985a)	<i>Echinochloa crus-galli</i>	GC	1	48	*		
	<i>Eleusine indica</i>	GC	1	48	*		
Read <i>et al.</i> (1997)	<i>Bouteloua gracilis</i>	GC	6	49	*		
Sionit & Patterson (1984)	<i>Digitaria sanguinalis</i>	GC	1	22			*
	<i>Echinochloa crus-galli</i>	GC	1	22			*
	<i>Eleusine indica</i>	GC	1	22			*
	<i>Setaria faberii</i>	GC	1	22			*
Sionit & Patterson (1985)	<i>Digitaria sanguinalis</i>	GC	2	43			*
	<i>Echinochloa crus-galli</i>	GC	2	43			*
	<i>Eleusine indica</i>	GC	2	43			*
	<i>Setaria faberii</i>	GC	2	43			*
Thompson & Drake (1994)	<i>Spartina patens</i>	OTC	G	1600			
Volin & Reich (1996)	<i>Bouteloua curtipendula</i>	GC	2.5	58			
Wand <i>et al.</i> (1996)	<i>Themeda triandra</i>	OTC	3.9	210			
Wilsey <i>et al.</i> (1994)	<i>Sporobolus kentrophyllus</i>	GC	4.2	42			
Wilsey <i>et al.</i> (1997)	<i>Sporobolus kentrophyllus</i>	GC	2	75			
	<i>Paspalum dilatatum</i>	GC	2	75			
	<i>Digitaria macroblephara</i>	GC	2	75			
	<i>Themeda triandra</i>	GC	2	75			
Wray & Strain (1986)	<i>Andropogon virginicus</i>	GC	0.5	56			*
Wray & Strain (1987)	<i>Andropogon virginicus</i>	GC	0.5	63			
Ziska <i>et al.</i> (1990)	<i>Spartina patens</i>	OTC	G	500			