

Elevated CO₂ enhances water relations and productivity and affects gas exchange in C₃ and C₄ grasses of the Colorado shortgrass steppe.

JACK A. MORGAN*, DANIEL R. LECAIN*, ARVIN R. MOSIER† and DANIEL G. MILCHUNAS‡

*USDA-ARS, Rangeland Resources Research Unit Crops Research Lab, 1701 Centre Ave., Fort Collins, CO 80526, USA;

†USDA-ARS, Soil, Plant Nutrient Research Unit, Federal Bldg., 301 S. Howes Street, Room 407, PO Box E, Fort Collins,

CO80522, USA and ‡Dept. of Rangeland Ecosystem Science and Natural Resources Ecology Lab, Colorado State University, Fort Collins, CO80523, USA

Abstract

Six open-top chambers were installed on the shortgrass steppe in north-eastern Colorado, USA from late March until mid-October in 1997 and 1998 to evaluate how this grassland will be affected by rising atmospheric CO₂. Three chambers were maintained at current CO₂ concentration (ambient treatment), three at twice ambient CO₂, or approximately 720 μmol mol⁻¹ (elevated treatment), and three nonchambered plots served as controls. Above-ground phytomass was measured in summer and autumn during each growing season, soil water was monitored weekly, and leaf photosynthesis, conductance and water potential were measured periodically on important C₃ and C₄ grasses. Mid-season and seasonal above-ground productivity were enhanced from 26 to 47% at elevated CO₂, with no differences in the relative responses of C₃/C₄ grasses or forbs. Annual above-ground phytomass accrual was greater on plots which were defoliated once in mid-summer compared to plots which were not defoliated during the growing season, but there was no interactive effect of defoliation and CO₂ on growth. Leaf photosynthesis was often greater in *Pascopyrum smithii* (C₃) and *Bouteloua gracilis* (C₄) plants in the elevated chambers, due in large part to higher soil water contents and leaf water potentials. Persistent downward photosynthetic acclimation in *P. smithii* leaves prevented large photosynthetic enhancement for elevated CO₂-grown plants. Shoot N concentrations tended to be lower in grasses under elevated CO₂, but only *Stipa comata* (C₃) plants exhibited significant reductions in N under elevated compared to ambient CO₂ chambers. Despite chamber warming of 2.6 °C and apparent drier chamber conditions compared to unchambered controls, above-ground production in all chambers was always greater than in unchambered plots. Collectively, these results suggest increased productivity of the shortgrass steppe in future warmer, CO₂ enriched environments.

Keywords: *Bouteloua gracilis*, *Pascopyrum smithii*, global change, photosynthesis, acclimation, grazing, defoliation

Received 14 June 2000; revised version received 8 October and accepted 18 October 2000

Introduction

Atmospheric CO₂ concentrations have increased from approximately 280 μmol mol⁻¹ in the late 19th century to over 360 μmol mol⁻¹ today, and are projected to double over present ambient concentrations by the mid- to late-

21st century (Alcamo *et al.* 1996). Numerous studies have been conducted to determine how CO₂ will affect crops as well as natural ecosystems and their dominant species. The vast majority of these studies have been conducted with C₃ plant species since C₃ photosynthesis is unsaturated at present atmospheric CO₂ concentrations (Stitt 1991), and most evidence suggests that growth of C₃

Correspondence: fax: 970-482-2909, e-mail: morgan@lamar.colostate.edu

plants responds more to CO₂ enrichment than C₄ species. C₄ photosynthesis is believed to be CO₂-saturated at present atmospheric CO₂ concentrations due to a highly efficient CO₂ pump that elevates CO₂ in the bundle sheath cells (Bowes 1993). However, significant CO₂-induced growth enhancements have been observed for many C₄ species, and their sensitivity to CO₂ is often similar to that observed in C₃ plants (Poorter 1993; Hunt *et al.* 1996; Poorter *et al.* 1996; Wand *et al.* 1999; Ghannoum *et al.* 2000).

Increasing CO₂ causes stomates of most species to close, resulting in increased water use efficiency (Drake *et al.* 1996; Kirkham *et al.* 1991; Nie *et al.* 1992). This water-relations enhancement from CO₂ helps explain the growth enhancement of C₄ plants, especially in water-limited systems (Owensby *et al.* 1996, 1999; Ghannoum *et al.* 2000). Improved plant water status from CO₂ enrichment may also stimulate C₄ (and C₃) plant growth by enhancing leaf expansion (Wand 1999). Although C₄ photosynthesis is nearly saturated at present atmospheric CO₂ concentrations, recent studies indicate that carbon fixation is sometimes enhanced in C₄ grasses with a doubling of CO₂ concentration, especially under well-illuminated conditions (Sionit & Patterson 1984; Morgan *et al.* 1994a; Ghannoum *et al.* 1997; Ziska & Bunce 1997; LeCain & Morgan 1998; Wand 1999; Wand *et al.* 1999). Therefore, it appears that CO₂ enhances growth in C₄ species primarily through improved water relations, and secondarily through photosynthetic enhancement.

While productivity and species composition of native grasslands are influenced by animal grazing (Milchunas & Lauenroth 1993), few field studies have explicitly evaluated the interaction of defoliation with CO₂ enrichment (Hebeisen *et al.* 1997; Owensby *et al.* 1999). The growth enhancing effect of elevated CO₂ is typically greatest in young plants, due to the high photosynthetic efficiency of young leaves, and also to the presence of strong sinks for assimilates (Baxter *et al.* 1995); these attributes may also enhance CO₂-induced photosynthetic and re-growth responses of young leaves in recently defoliated canopies. However, repeated and/or severe defoliations may eliminate yield response to CO₂ in the present growing season (Hebeisen *et al.* 1997) or weaken plants and reduce regrowth in subsequent growing seasons (Trlica *et al.* 1977; Menke & Trlica 1983), which presumably could affect their long-term responsiveness to CO₂.

In water-limited grasslands, the effects of elevated CO₂ on regrowth often involve interactions with water. Owensby *et al.* (1999) observed that in C₄-dominated tallgrass prairie, CO₂ enhanced regrowth when late-season water stress occurred, presumably because of the enhanced water use efficiency. In a subsequent year when late-season conditions were wetter, no such CO₂

enhancement of regrowth was observed. Defoliation may temporarily reduce canopy-level transpiration, thereby improving water use efficiency (Milchunas *et al.* 1995) and possibly interacting with the effect of elevated CO₂ on water relations.

The shortgrass steppe is a semiarid grassland along the western edge of the Great Plains of the United States, stretching from south-eastern New Mexico and western Texas north to the Colorado-Wyoming border at 41°N latitude (Lauenroth & Milchunas 1991). Vegetation of this region is dominated by warm-season, C₄ grasses (*Bouteloua* and *Buchloe* spp.), but contains an abundance of cool-season, C₃ grasses (e.g. *Pascopyrum* and *Stipa* spp.), as well as a variety of C₃ forbs and woody vegetation; C₄ forbs and woody vegetation are uncommon. Previous CO₂ enrichment studies in North American grasslands have been conducted on tallgrass prairie in Kansas (Kirkham *et al.* 1991; Nie *et al.* 1992; Owensby *et al.* 1993b, 1996) and annual grassland in California (Chiariello & Field 1996). Only one has considered how defoliation might affect the response of grasses to CO₂ (Owensby *et al.* 1999).

This study was undertaken to evaluate how doubling the CO₂ concentration influences growth of important C₃ and C₄ species in the shortgrass steppe of eastern Colorado, USA. Based on previous controlled-environment work with these species (Hunt *et al.* 1996; Morgan *et al.* 1998) we hypothesized similar and substantial growth enhancement with elevated CO₂. Two defoliation regimes were initiated to simulate grazing and to determine how CO₂ enrichment interacts with defoliation. We hypothesized that the growth-enhancing effects of elevated CO₂ would be greater in summer-defoliated plots compared to plots which remained un-defoliated during the growing season. Measurements of photosynthesis and water potential were obtained at the leaf level to evaluate how long-term exposure to high CO₂ affected basic plant physiological traits in individual C₃ and C₄ grasses. An important goal was to determine whether photosynthetic acclimation measured previously in growth chamber studies of these grasses (Morgan *et al.* 1994a; Read *et al.* 1997; LeCain & Morgan 1998) also occurred in the field.

Materials and methods

The study site is at the USDA-ARS Central Plains Experimental Range (CPER), lat. 40°40' N, long. 104°45' W, in the shortgrass steppe region of north-eastern Colorado (Lauenroth & Milchunas 1991), about 56 km north-east of Fort Collins, CO. Long-term (55 year) mean annual precipitation averaged 320 mm, with the majority occurring during May, June and July. Mean air temperatures are 15.6°C in summer and 0.6°C in winter with

maximum July temperatures averaging 30.6°C. Basal cover is 25–35% of which up to 90% is *Bouteloua gracilis* (H.B.K.) Lag. (blue grama), a warm season, C₄ grass. In some areas the cool season, C₃ grasses *Pascopyrum smithii* (Rydb.) A. Love (western wheatgrass) and *Stipa comata* Trin and Rupr. (needle-and-thread grass) are also a major vegetation component. The soil at the experimental site is a Remmit fine sandy loam (Ustollic camborthids). This sandy soil holds 18% water at field capacity, and 4% at the permanent wilting point.

The effect of elevated CO₂ on this native ecosystem was investigated using open top chambers (4.5 m diameter, enclosing 15.5 m²). The experiment was established on a six ha native rangeland pasture with a mixture of C₃ and C₄ grass species. Prior to 1996 the field had been grazed by cattle at a light to moderate intensity (about 30% annual forage removal). A portion of the pasture was initially divided into three blocks based on uniformity of vegetation, and three 15.5 m² circular plots per block were randomly chosen as experimental plots. From late March until mid-October in 1997 (March 20 and October 18) and 1998 (March 24 and October 13), open top chambers were placed on two plots in each of the three blocks (six total). One chamber was randomly assigned an ambient CO₂ treatment (360 ± 20 μmol mol⁻¹), the other an elevated CO₂ treatment (720 ± 20 μmol mol⁻¹). Carbon dioxide fumigation proceeded as soon as the chambers were placed on the plots, and continued until they were removed in the autumn. Each block also had an unchambered plot of equal ground area, which was used to monitor the effect of the chamber.

Chambers were constructed with six 3.8 m high by 2.5 m wide walls made with a Unistrut galvanized steel tubing frame (Unistrut Corp. Wayn, MI, USA) covered with clear, Lexan* (Regal Plastics, Littleton, CO, USA) panels. The top was covered with a Unistrut and Lexan frustrum, reducing the opening to 0.75 m diameter. Chambers were aspirated with outside air by large fans, delivering air through 1.14 m² inlet ports (located 0.16 m above the soil surface) on three sides of the chamber, which achieved an air exchange rate of approximately one-and-a-half chamber volumes per min. An outlet fan in the top of the chambers equilibrated the pressure inside and outside the chambers. Soil within the chambers was isolated from the outside by an aluminium flange buried 0.8 meter deep around the outside edge of the chamber wall in early 1996. CO₂ concentration within

the chambers was monitored by drawing air from a sampling manifold, positioned 2.5 m high and in the centre of each chamber, to an infra-red gas analyser (LI-COR LI6262; LI-COR, Lincoln, NE, USA). CO₂ was elevated in three of the chambers by injecting pure CO₂ (Air Liquide America Corp., Denver, CO, USA) into the chamber inlet air stream. In 1998 air temperature (thermocouples positioned 15 cm above the soil) and light flux (LI-COR LI-190SA) were monitored in the chambered and unchambered plots throughout the growing season. Chambers were removed in the winter when vegetation is dormant.

Precipitation was returned to the chambered plots with an automatic system. The outer edge of the frustrum has a rain gutter which captures the precipitation and drains it to a barrel. A float switch in the barrel activates a water pump which evenly distributes the water through a sprinkler system inside the chambers. The precipitation catching system is not 100% efficient, so the amount of applied water was carefully monitored and deficiencies were made up at least weekly.

The south half of the ground area within the chambers was designated for plant harvests. A metal wire grid was made which contained 56 40.5 × 15.3 cm quadrats (3.46 m² total). During the period of peak standing crop (late July) this grid was placed over the south half of each plot and vegetation in every other quadrat was clipped to the crown, separated by species, dried at 60°C and weighed. Sub-samples of *B. gracilis*, *P. smithii* and *S. comata* shoots from this harvest were fine-ground, weighed and analysed for total N with a combustion C and N analyser. After the chambers were removed in mid-October, senescent phytomass from the summer-defoliated quadrats was harvested for regrowth. The other remaining 28 quadrats were harvested in a single autumn clipping to estimate seasonal productivity for plants which remained nondefoliated during the growing season. The pattern was reversed the following year; quadrats which had not been clipped the previous summer were clipped at peak standing crop as well as in autumn, and the alternate quadrats harvested only in the autumn. This defoliation protocol, which removed 50% of the green vegetation and represents defoliation by cattle that is between moderate and heavy grazing practices, was incorporated into the CO₂-treatment experimental design primarily as a means to represent the nominal grazed conditions for these grasslands (Klippel & Costello 1960; Milchunas *et al.* 1988, 1995). Secondly, the defoliations were designed to allow an assessment of the effects of CO₂ on the capacity of the plants to regrow following current-year defoliation. Annual alternation of the small grids that received or did not receive the defoliation during a particular growing season allowed for cycles of 'rest-from-defolia-

*Mention of a trademark or manufacturer by the USDA or Colorado State University does not imply its approval to the exclusion of other products of manufactureres that may also be suitable.

tion'. The October final harvest involved only senescent shoot material, and so did not elicit a physiological response. Phytomass in the north half of the chambers was defoliated close to the crown (about 2 cm height) in October so that plant cover throughout the chamber was comparable at the beginning and end of each growing season. Thirty-six species were found in the chambers, but phytomass data were pooled into three functional groups for this report; C₃ grasses, C₄ grasses and forbs.

Soil moisture was measured weekly using a Troxler model 4301 neutron probe (Troxler Electronics Laboratory, Research Triangle Park, NC, USA) which had been calibrated against soil from the experimental site. Soil moisture content was converted to the total mm of water in the upper meter of the soil profile.

Leaf water potential was measured weekly between 10 and 11:45 am (Mountain Savings Time) on three of the major species, *B. gracilis*, *P. smithii* and *S. comata*, using a PMS pressure chamber (Plant Measurement Systems, Corvallis, OR, USA). Two recently expanded leaves were measured from each species in each plot and the data averaged. Sample leaves were taken from plants in the northern half of the chambers to avoid disturbing the area reserved for plant harvests.

CO₂ exchange rate (CER) and leaf conductance were measured about every three weeks on *B. gracilis* and *P. smithii* using the CIRAS-1 steady state, portable gas analysis system with a PLC (N) leaf chamber (PP systems, United Kingdom). CER was measured in the northern half of each plot on two to four newly expanded leaves of each species at about 360 and 720 $\mu\text{mol mol}^{-1}$ CO₂. The leaves were always selected at the top of a shoot, and because of the low leaf area of the grassland, were well-illuminated prior to measurement. The photosynthesis system provides temperature and vapour pressure control, which were set near ambient conditions on the day of measurement, and has a light unit which provides 1300 $\mu\text{mol m}^{-2}\text{s}^{-1}$ quanta. The area of leaves within the cuvette was measured and CER expressed on a leaf area basis.

Above-ground standing phytomass data were analysed using the SAS (SAS Institute Inc., Cary, NC) PROC anova procedure. We assumed a randomized block design. Main effects included CO₂ (includes chambered and nonchambered) and year, and one analysis explicitly evaluated defoliation as a third treatment. In an analysis of variance for the combined annual accrual of phytomass on autumn-only plus summer/autumn clipped quadrats, defoliation was not analysed as a separate treatment; the alternate-year clipping scheme was instituted so productivity responses to elevated CO₂ could be evaluated in a grassland in which animal grazing was being simulated. However, defoliation was treated as a separate treatment in another analysis of variance which

compared accrual of phytomass in autumn vs. summer/autumn-defoliated quadrats, to evaluate the defoliation response in addition to year and CO₂ effects. Significant year and year by CO₂ treatment interactions were observed for summer above-ground phytomass and also for the analyses involving defoliation responses, so means comparisons of all harvest data were presented within year. Means of three replications are shown with Fishers Least Significant Difference means comparison test when the probability that the null hypothesis is true is less than 5%. Soil moisture, leaf water potential and leaf CER are presented in figures as means of three replications \pm standard errors.

Results

1996. Before CO₂ Enrichment. One year prior to the initiation of chamber/CO₂ treatments, above-ground phytomass was sampled in mid-July for baseline plant community information (Fig. 1). This summer harvest date is the approximate time at which peak standing phytomass occurs in the shortgrass steppe of eastern Colorado. We detected no significant differences in above-ground phytomass (115 g/m²) in the three treatment areas, nor in the percentages of functional groups represented in the above-ground phytomass (54.4% C₃ grasses, 44.4% C₄ grasses, and 1.2% forbs). However,

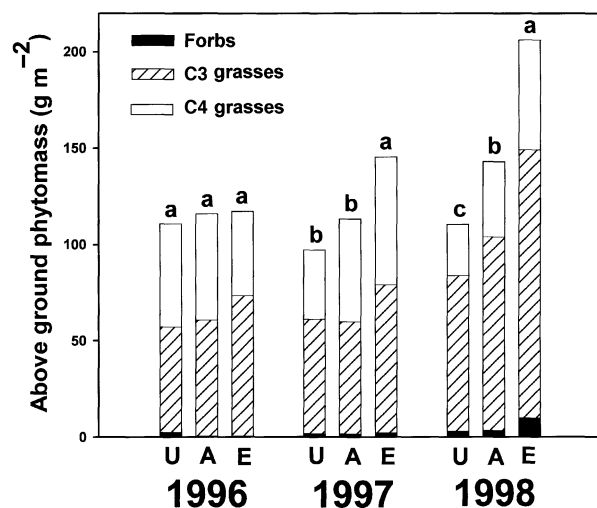


Fig. 1 Total standing above-ground phytomass of C₃ grasses, C₄ grasses and forbs harvested in mid-July of 1996 (pre-CO₂ treatment) 1997 and 1998 in unchambered (U), ambient (A) and elevated (E) CO₂ open-top chamber plots. Ambient chambers had 360 \pm 20 and elevated chambers had 720 \pm 20 $\mu\text{mol mol}^{-1}$ CO₂ and unchambered were plots of equal ground area with no chamber on them. Data are means of three replications. Means with different letters per year are significantly different at $P < 0.05$.

there was a slight trend ($P=0.26$) suggesting the elevated plots contained 10–12% more C₃ and 10–11% less C₄ grass above-ground phytomass compared to the ambient and unchambered plots. Therefore, we used these baseline percentages as covariates in all statistical analyses conducted in subsequent years for determining the percentage of functional groups in above-ground phytomass.

Precipitation and Chamber Conditions. The two years of the study were wetter than long-term averages, with 562 and 422 mm of rainfall falling in 1997 and 1998, respectively, compared to 320 mm/year averaged over the past 55 years. The distribution of rainfall during the growing seasons is shown in Fig. 2. Thermocouples placed at canopy height in 1998 indicated that, on average, within chamber air temperatures were 2.6 °C warmer compared to unchambered plots. We could not detect a temperature differential between the ambient and elevated chambers. Quantum sensors placed inside the chambers indicated that greater than 95% of incident photosynthetic active radiation was transmitted through

the Lexan chamber walls. However, shading by chamber framework reduced the daily photon flux to 28% less inside compared to outside the chambers.

Above-ground Phytomass Responses to CO₂. An analysis of variance revealed significant year and year by treatment interactions for standing above-ground phytomass determined in quadrats harvested in mid-July, so data were analysed and presented for each year separately (Fig. 1). In both years, significant treatment effects were observed, with elevated CO₂ plants having greater above-ground production by July compared to plants in ambient and unchambered plots. Above-ground phytomass in elevated plots was 27% greater in 1997 and 43% greater in 1998 compared to ambient plots. In 1998, phytomass was significantly less in the unchambered compared to both ambient and elevated plots. A covariance analysis of treatment effects on percentages of C₃/C₄ or forbs revealed no significant treatment differences in either year.

Peak standing above-ground phytomass was greater in 1998 than 1997 (Fig. 1). The balance between functional

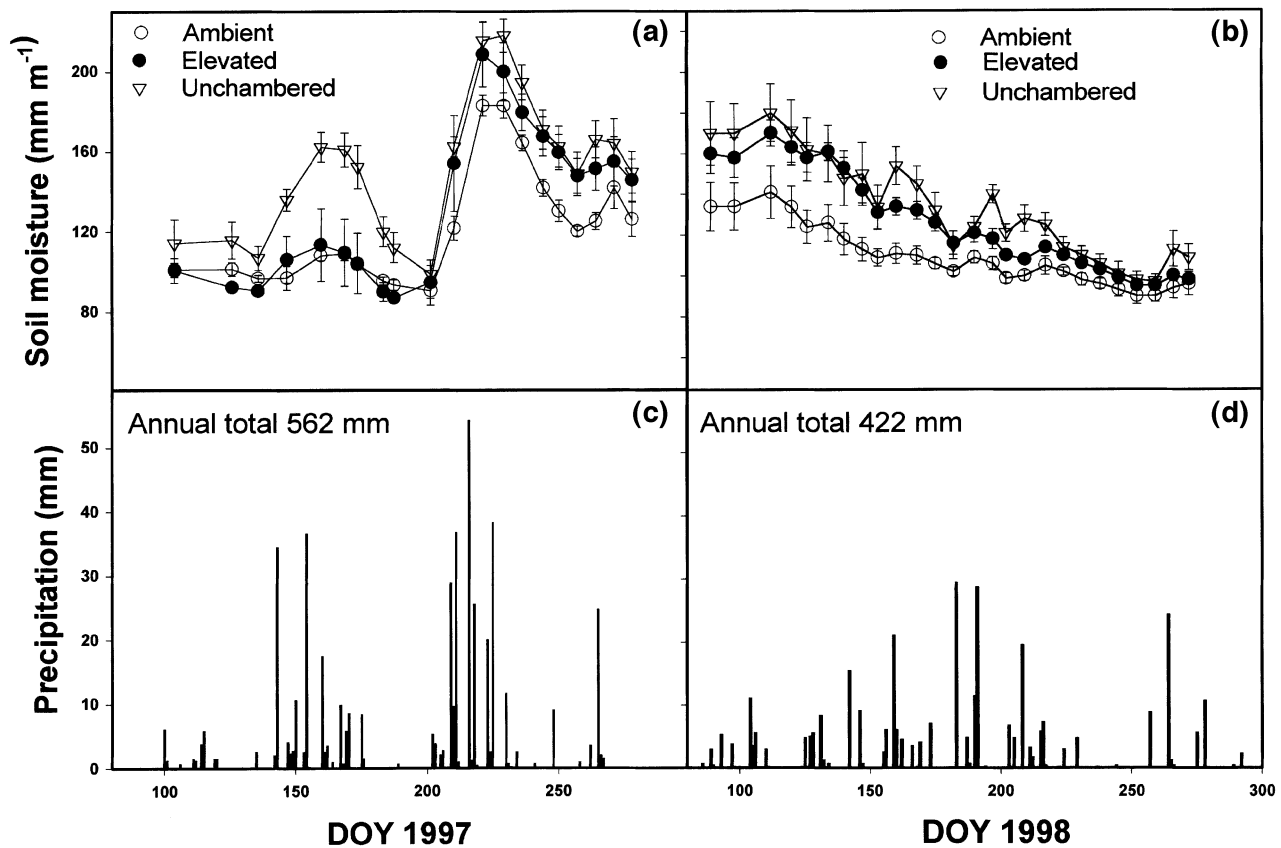


Fig. 2 Precipitation and soil moisture at the open-top-chamber experiment in 1997 and 1998. Ambient chambers had 360 ± 20 and elevated chambers had $720 \pm 20 \mu\text{mol mol}^{-1}$ CO₂ and unchambered were plots of equal ground area with no chamber on them. Soil moisture was measured with a neutron probe on three replicate chambers and water content was totaled for the upper meter of soil. Bars are standard errors.

Table 1 Autumn re-growth phytomass (Nov. 3 in 1997 and Oct. 15 in 1998) and annual accrual of above-ground phytomass* in ambient and elevated (360 and 720 $\mu\text{mol mol}^{-1}$) CO_2 open-top-chambers and in unchambered plots. Analysis of variance *P*-values are presented for CO_2 and year effects on above-ground phytomass.

1997		
Above-ground Phytomass		
Treatment	Autumn re-growth of 28 summer-clipped quadrats	Annual accrual for 28 summer/autumn-clipped quadrats + 28 autumn-clipped quadrats (averaged over all 56 quadrats)
g m^{-2}		
Unchambered	40.7 c	105.9 c
Ambient CO_2	74.2 b	152.7 b
Elevated CO_2	93.4 a	198.0 a
<i>P</i> > <i>f</i>	0.006	0.013

1998		
Above-ground Phytomass		
Treatment	Autumn re-growth of 28 summer-clipped quadrats	Annual accrual for 28 summer/autumn-clipped quadrats + 28 autumn-clipped quadrats (averaged over all 56 quadrats)
g m^{-2}		
Unchambered	26.6 c	105.9 c
Ambient CO_2	48.8 b	163.3 b
Elevated CO_2	71.9 a	246.8 a
<i>P</i> > <i>f</i>	0.008	0.002

Analysis of Variance <i>P</i> Values for CO_2 and Year Effects on Above-ground Phytomass		
Source	Autumn re-growth of 28 summer-clipped quadrats	Annual Accrual averaged over all 56 quadrats
block	NS	NS
CO_2	0.005	0.003
block * CO_2	NS	NS
year	0.003	0.046
CO_2 * year	NS	NS

*Above-ground phytomass data are means of three replications. Probabilities of a significant CO_2 treatment effect are given within each year. Fisher's least significant difference means comparison test is shown. Means followed by different letters are statistically different at the $P \leq 0.05$ level.

groups also was different between years, with C_3 grasses being more prominent and C_4 grasses less prominent in 1998 compared to 1997.

Analysis of variance for autumn re-growth of summer-clipped quadrats, and total annual accrual of phytomass on combined summer/autumn and autumn-only clipped quadrats (an estimate of annual phytomass accrual under moderate to heavy grazing) indicated significant CO_2 treatment and year effects (Table 1). Separation of vegetation into functional groups was not done with the autumn-harvested material because vegetation was

senescent and difficult to discriminate among species. In comparisons between ambient and elevated chambers, autumn re-growth phytomass was 26% greater in 1997 and 47% greater in 1998 under elevated CO_2 . Responses of total annual accrual of phytomass across all plots to CO_2 were very similar to the summer harvested (Fig. 1) and autumn re-growth results, with CO_2 enrichment increasing annual above-ground phytomass accrual in elevated compared to ambient chambers 30% in 1997 and 47% in 1998. In both years, growth of unchambered vegetation was less than that of chambered vegetation.

Table 2 Annual accrual of standing above-ground phytomass* in 28 quadrats clipped once in the autumn vs. 28 other quadrats clipped twice, in summer and autumn, in ambient and elevated (360 and 720 $\mu\text{mol mol}^{-1}$) CO₂ open-top-chambers and in unchambered plots. Quadrats were harvested in summer (July 18 in 1997 and July 22 in 1998) in both years and again in autumn (Nov. 3 in 1997 and Oct. 15 in 1998). Anova *P*-values are presented for CO₂, year and defoliation effects on above-ground phytomass.

1997		
Above-ground Phytomass		
Treatment	One Clipping autumn (28 quadrats samples once)	Two Clippings summer and autumn re-growth (28 quadrats samples twice)
	g m^{-2}	
Unchambered	78.0 b	133.9 c
Ambient CO ₂	116.3 ab	189.0 b
Elevated CO ₂	155.0 a	241.3 a
<i>P</i> > <i>f</i>	0.029	0.007
1998		
Above-ground Phytomass		
Treatment	One Clipping autumn (28 quadrats samples once)	Two Clippings summer and autumn re-growth (28 quadrats samples twice)
	g m^{-2}	
Unchambered	85.5 c	133.4 c
Ambient CO ₂	143.1 b	193.5 b
Elevated CO ₂	213.9 a	279.6 a
<i>P</i> > <i>f</i>	0.002	0.005

ANOVA *P* Values for CO₂, Year and Defoliation Effects on Above-ground Phytomass

block	NS
CO ₂	0.003
year	0.001
defoliation	0.001
CO ₂ * year	0.017
CO ₂ * defoliation	NS
year * defoliation	NS
CO ₂ * year * defoliation	NS

*Data are means of three replications. Probabilities of a significant treatment effect are given within each year. Means followed by different letters are statistically different at the $P \leq 0.05$ level (Fisher's LSD).

As with summer clipping (Fig. 1), phytomass was greater in 1998 compared to 1997.

Interaction of Defoliation and CO₂. To evaluate the interactive effect of defoliation and CO₂, an analysis of variance was conducted wherein we compared seasonal phytomass accrual of the 28 summer/autumn defoliated quadrats vs. the remaining 28 quadrats which were clipped only once in autumn for the final phytomass harvest (Table 2). In this analysis, defoliation was explicitly considered, and appeared in the analysis results as a treatment response. The results indicated

significant effects of CO₂, year and defoliation on phytomass, as well as a CO₂ treatment by year interaction. Seasonal phytomass accrual was greater for CO₂-enriched quadrats as well as for quadrats defoliated in summer (and autumn). The interaction between CO₂ and year was due to a greater response in the second year, although the greater second year responses was due in large part to a greater difference between the unchambered and chambered plots. Most interesting was the absence of a significant interactive effect of defoliation on the CO₂ growth response, as indicated by the analysis of

Table 3 Shoot nitrogen concentration of *Bouteloua gracilis*, *Pascopyrum smithii* and *Stipa comata* grown in ambient and elevated CO₂ open-top chambers and unchambered plots. Plants were harvested near the time of peak standing crop (late July) in 1996, 1997 and 1998.*

1996			
CO ₂ treatment	<i>B. gracilis</i>	<i>P. smithii</i>	<i>S. comata</i>
		N (g kg ⁻¹)	
Unchambered	11.6 ± 0.2	11.8 ± 0.7	11.3 ± 0.4
Ambient CO ₂	11.8 ± 0.2	11.5 ± 0.2	11.9 ± 0.1
Elevated CO ₂	12.6 ± 0.4	13.3 ± 0.2	12.0 ± 0.3
P > f	NS	NS	NS
1997			
CO ₂ treatment	<i>B. gracilis</i>	<i>P. smithii</i>	<i>S. comata</i>
		N (g kg ⁻¹)	
Unchambered	11.6 ± 0.3 a	11.1 ± 0.3	11.0 ± 0.3 a
Ambient CO ₂	10.5 ± 0.6 ab	11.6 ± 1.3	11.8 ± 0.5 a
Elevated CO ₂	8.9 ± 0.4 b	9.4 ± 0.9	8.5 ± 0.3 b
P > f	0.019	NS	0.0011
1998			
CO ₂ treatment	<i>B. gracilis</i>	<i>P. smithii</i>	<i>S. comata</i>
		N (g kg ⁻¹)	
Unchambered	11.3 ± 0.6	8.5 ± 0.1	8.7 ± 0.5 a
Ambient CO ₂	10.0 ± 0.2	9.5 ± 1.2	7.4 ± 0.6 ab
Elevated CO ₂	9.6 ± 0.4	7.8 ± 0.8	6.0 ± 0.5 b
P > f	NS	NS	0.028

*Data are means of three replications ± standard errors. Probabilities of a significant effect are given within year. Means followed by different letters are statistically different at $P \leq 0.05$ level (LSD).

variance which combined both years. There was also no significant interaction of defoliation with year or of defoliation with year and CO₂.

Summer Harvest Shoot N. Concentrations of N in shoot tissues of *B. gracilis*, *P. smithii* and *S. comata* were low, averaging 10.4 g/kg across all treatments, species and years (Table 3). Although trends were consistent suggesting lower N concentration in all grasses under elevated CO₂, only *S. comata* plants had significantly ($P < 0.05$) lower shoot N under elevated compared to ambient CO₂ chambers, and only in 1997.

Water Relations. During the first few months of CO₂ enrichment (1997), no clear effects of CO₂ on soil water content were detected. Near day-of-year (DOY) 145, several intense rain showers occurred (Fig. 2c), but only about half of the rainfall was returned to the chambers via the rain-catchment system. From these early experiences we learned that during intense rainfall, some of the precipitation bounces off the chambers and needs to be replaced with supplemental water. This was not done for this occasion, which resulted in a relatively dry period for the chambered plots compared to the unchambered plots (Fig. 2a). A subsequent period of rain shower activity which began at DOY 202 was followed by

treatment separation in soil water content, with the ambient plots losing water at a faster rate than in the elevated and unchambered plots. This resulted in lower soil water content in ambient plots by the end of the 1997 growing season that continued into the following year. Soil water content remained lower in ambient plots for most of 1998 (Fig. 2b), although by the end of the year soil water content had converged in all treatments. Throughout the two years, soil water contents of the unchambered plots were closer to values measured in elevated plots and almost always greater than that measured in the ambient plots, suggesting the chambers had a desiccating effect.

Measurements of leaf water potential (ψ) in 1997 and 1998 reflected, in large part, treatment differences in soil water content (Fig. 3). For *B. gracilis*, *P. smithii* and *S. comata*, ψ were generally greater in elevated compared to ambient plots. Periodic measurements of ψ in unchambered plots were often similar to those measured in ambient plots, despite higher soil water contents in unchambered plots.

Photosynthesis. In both years, seasonal changes in precipitation and soil water content were reflected in leaf photosynthetic activity. The mid-summer dry spell

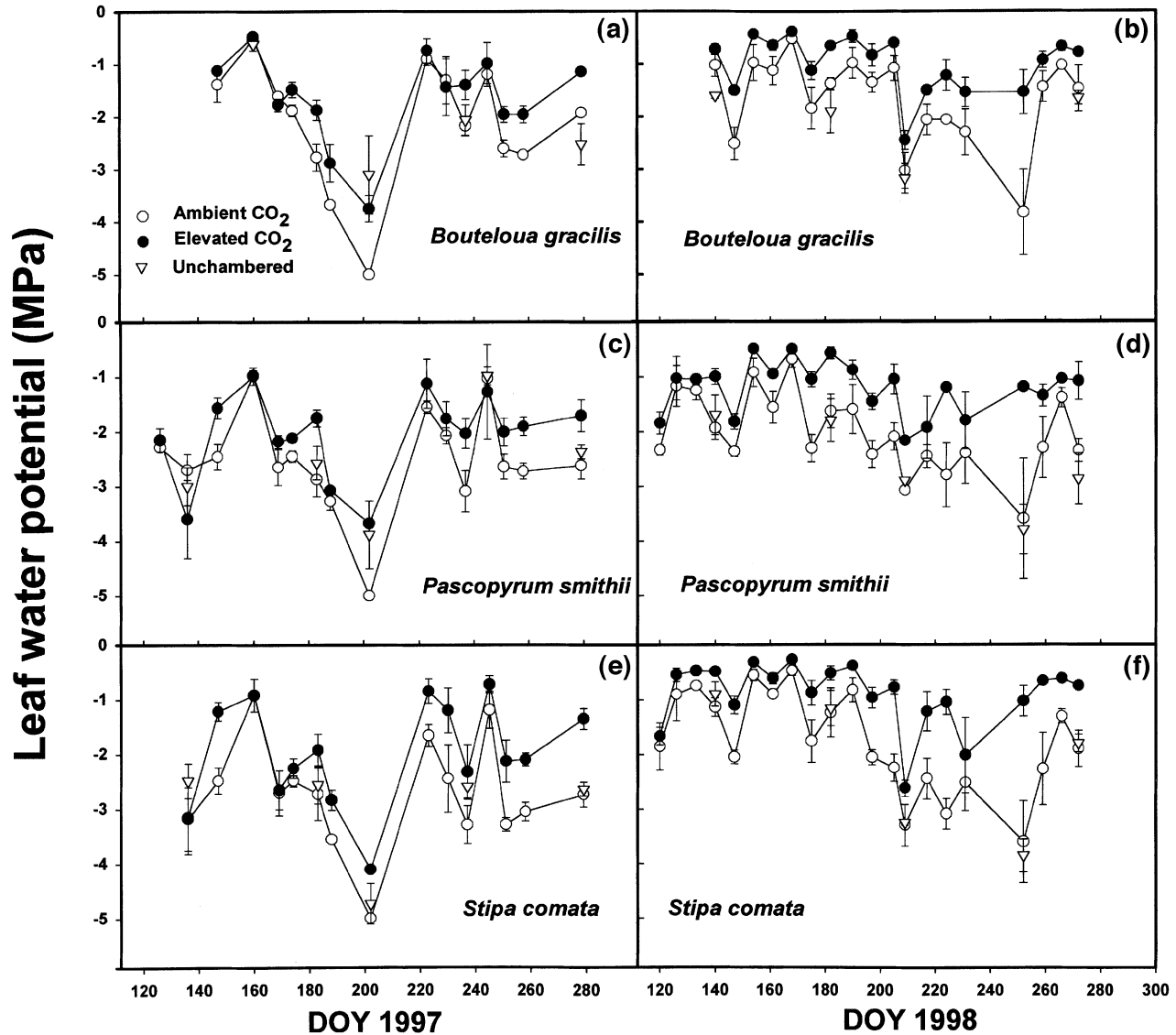


Fig. 3 Leaf water potential of *Bouteloua gracilis* (a,b), *Pascopyrum smithii* (c,d) and *Stipa comata* (e,f) at the open-top-chamber experiment in 1997 and 1998. Ambient chambers had 360 ± 20 and elevated chambers had $720 \pm 20 \mu\text{mol mol}^{-1}$ CO₂ and unchambered were plots of equal ground area with no chamber on them. Leaf water potential was measured with a pressure chamber on two leaves from each of three replicate chambers per treatment. Bars are standard errors.

in 1997 was evident in CERs of both *B. gracilis* and *P. smithii*, which declined from DOY 160–205, and then rose again in response to precipitation after DOY 204 (Fig. 4a and 4c). Similarly, CERs exhibited maximal or near-maximal rates at the beginning of the growing season in 1998, when soil water contents were highest for the year, and then declined in concert with declining soil water content through the growing season (Fig. 2,4b,d).

The data show that for both species, CERs measured under chamber conditions were either no different in ambient and elevated chambers, or were occasionally higher for plants grown under elevated CO₂ (Fig. 4).

Under chamber conditions, photosynthesis was enhanced no more by elevated CO₂ in the C₃ grass *P. smithii* than in the C₄ grass, *B. gracilis*. Elevated CO₂ reduced stomatal conductance in both species in 1997, although this response was barely evident in 1998 (Fig. 5).

When comparisons were made between CER measurements conducted at the same CO₂ concentration (either 360 or 720 $\mu\text{mol mol}^{-1}$) on leaves grown at ambient or elevated CO₂, different results were observed for *B. gracilis* and *P. smithii*. No consistent treatment effect of growth CO₂ concentration was observed for *B. gracilis*

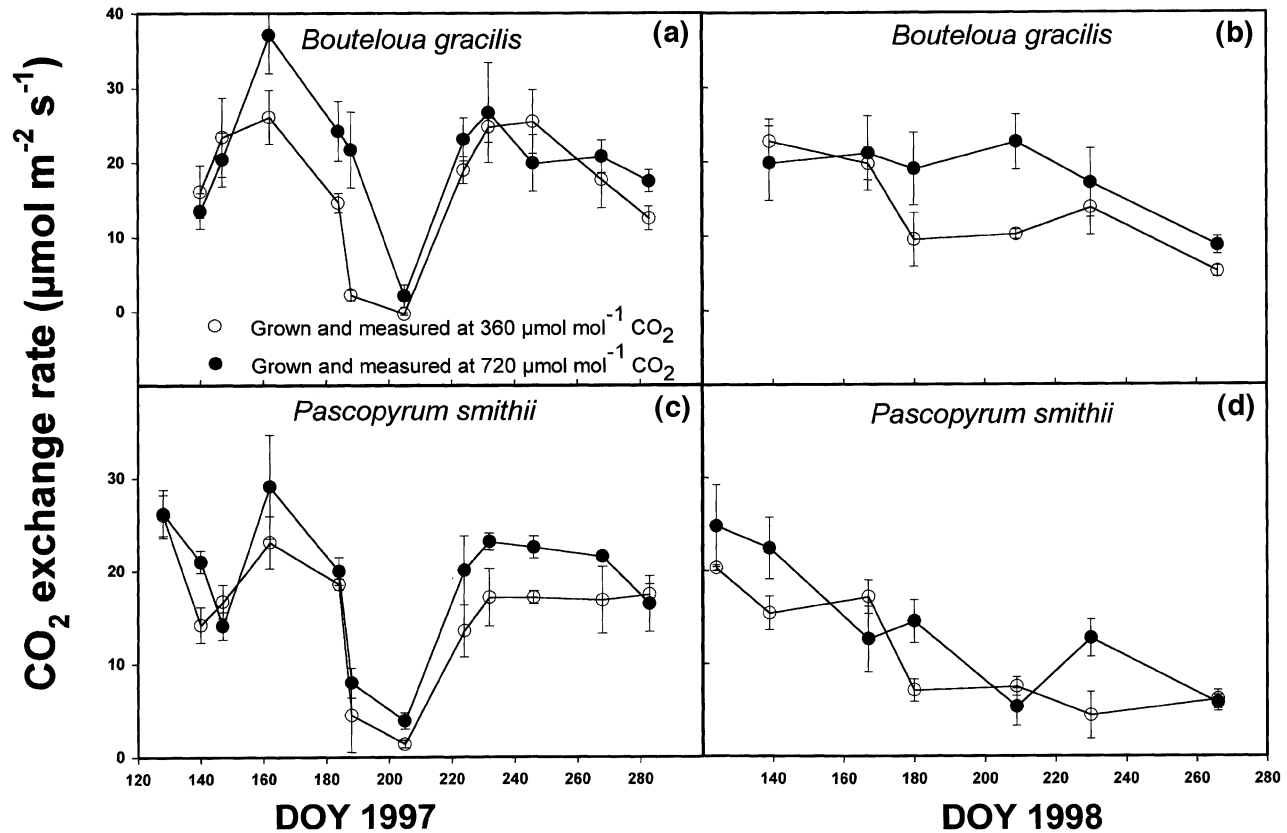


Fig. 4 Leaf CO₂ exchange rate of *Bouteloua gracilis* (a,b), and *Pascopyrum smithii* (c,d) at the open-top-chamber experiment in 1997 and 1998 conducted under chamber conditions of CO₂. Gas exchange was measured at leaf chamber CO₂ concentrations equal to that at which the plants were grown: ambient (360 ± 20 µmol mol⁻¹) or elevated (720 ± 20 µmol mol⁻¹). Bars are standard errors.

leaves measured at 360 (Figs 6a and 7a) or 720 (Figs 6b and 7b) µmol mol⁻¹. Photosynthesis rates were slightly greater when measured at 720 (Figs 6b and 7b) compared to 360 (Figs 6a and 7a) µmol mol⁻¹ CO₂, indicating a small direct photosynthetic response of *B. gracilis* in this study. In contrast to *B. gracilis*, CERs of *P. smithii* leaves were considerably higher when measured at elevated (Figs 6d and 7d) compared to ambient CO₂ (Figs 6c and 7c), indicating a significant short-term photosynthetic response to CO₂. However, when measurements were conducted at the same CO₂ concentration, CERs of leaves grown under elevated CO₂ were generally lower, but sometimes no different from ambient-grown plants (Figs 6c,d and 7c,d).

Discussion

Above-ground Production. This study conducted in the shortgrass steppe of north-eastern Colorado, USA confirms what previously was determined in controlled environments (Hunt *et al.* 1996; Morgan *et al.* 1998); exposure of this semiarid grassland to 720 µmol mol⁻¹

CO₂ enhances plant productivity over present atmospheric CO₂ concentrations. The study focused on above-ground plant responses. Assuming phytomass measurements are reasonable estimates of above-ground production, the results of this study indicated that production under a defoliation regime which removes about 50% of the live vegetation in a growing season was stimulated 30% in 1997 and 47% in 1998. Previous controlled-environment studies with these species suggest that below-ground growth enhancements from CO₂ enrichment would be as great (Hunt *et al.* 1996; Morgan *et al.* 1998) or greater (Morgan *et al.* 1994b, 1998) than those measured above-ground. These results support theory and data which indicate significant CO₂-induced growth enhancements will be realized in water-limited environments due to increased water use efficiency under elevated CO₂ (Kirkham *et al.* 1991; Mooney *et al.* 1991; Nie *et al.* 1992; Owensby *et al.* 1993b, 1996; Koch & Mooney 1996; 1999). Percentage increases in tallgrass prairie production from elevated CO₂ ranged from approximately nil in years with plentiful moisture to 36% enhancement in dry years (Owensby *et al.* 1993b,

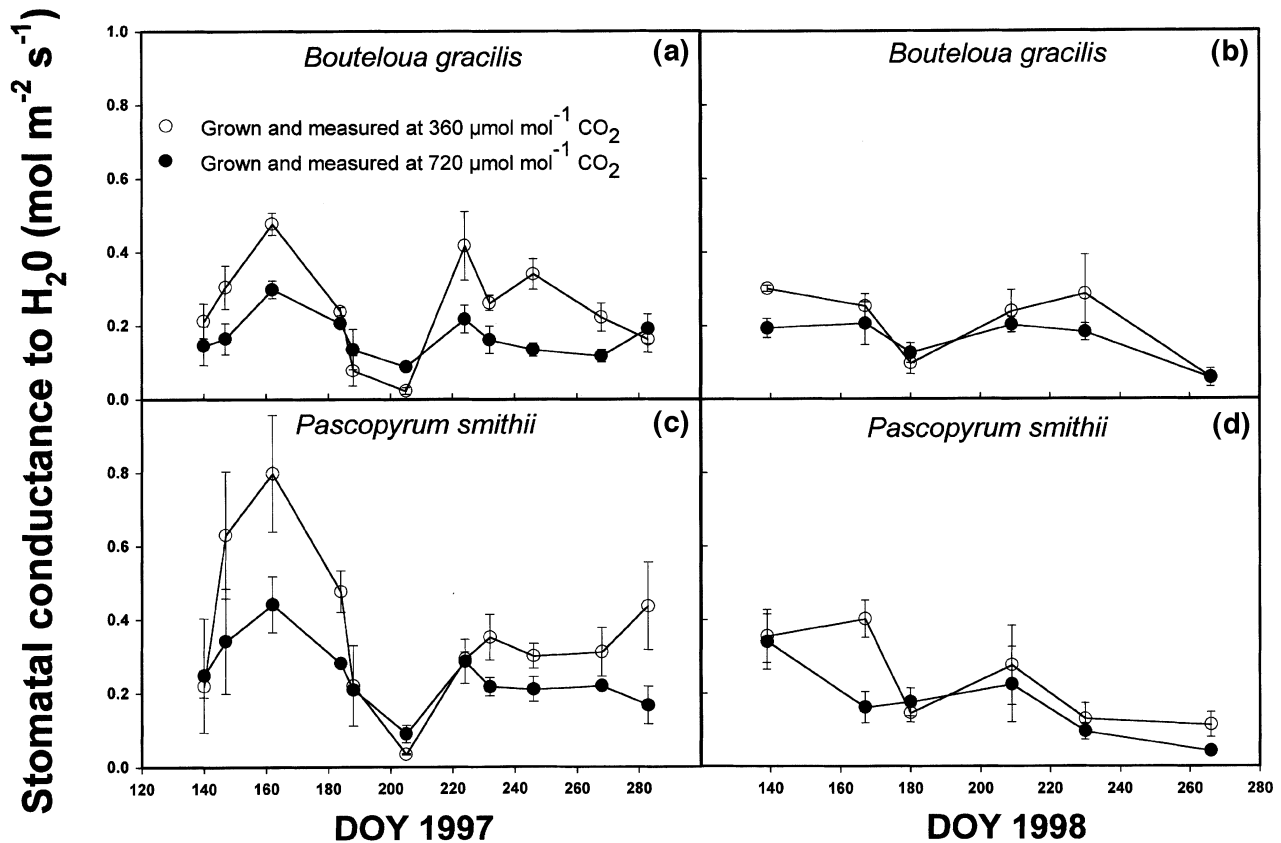


Fig. 5 Leaf stomatal conductance to water vapour of *Bouteloua gracilis* (a,b) and *Pascopyrum smithii* (c,d) at the open-top-chamber experiment in 1997 and 1998. Stomatal conductance was measured at a leaf chamber CO₂ concentration equal to that at which the plants were grown: ambient ($360 \pm 20 \mu\text{mol mol}^{-1}$) or elevated ($720 \pm 20 \mu\text{mol mol}^{-1}$). Bars are standard errors.

1996). Because water limitations are characteristic of the shortgrass steppe, CO₂-induced growth enhancements will likely be more consistent from year to year, as they were in this study and in previous growth chamber work with shortgrass steppe plants (Hunt *et al.* 1996).

In both years of the study, above-ground production was higher than is generally seen on the short-grass steppe, likely a result of greater than average precipitation in both years of the study. Although total precipitation was less in 1998, production and the proportion comprised of C₃ grasses was greater compared to 1997, perhaps due to a more uniform distribution of precipitation in 1998.

CO₂ and Defoliation. CO₂ enrichment enhanced production similarly regardless of whether plants were defoliated once in summer and then again in autumn after senescence, or only once at the end of the growing season. It is possible that the experimental design of this study involving defoliation (see Materials and Methods) could have precluded detecting a defoliation by CO₂ interaction. If the present season's defoliation scheme had a significant effect on regrowth the next year, then

that carry over effect could have confounded our treatment effects, and prevented detecting an interaction of defoliation with CO₂. However, the statistical results did not reveal any such carry over effect, as indicated by a lack of significance for defoliation by year or defoliation by year by CO₂ interactions for phytomass.

How grasslands respond to CO₂ enrichment under defoliation ultimately depends on the number and severity of defoliations as well as the ability to obtain soil resources (Hebeisen *et al.* 1997; Owensby *et al.* 1999). While our results were similar to previous controlled-environment studies in which no interactive effects of defoliation on CO₂-induced growth responses of grasses were detected (Wilsey *et al.* 1994, 1997; Wand 1999), we suspect the results of all these studies may not apply simply to all grasslands. In grasslands in which seasonal water supply often limits production, the timing of defoliation and its interaction with soil water will determine its relative CO₂ response. The conditions of our study were such that above-ground production was more than 50% greater in quadrats that were defoliated in summer. This compensatory response was probably

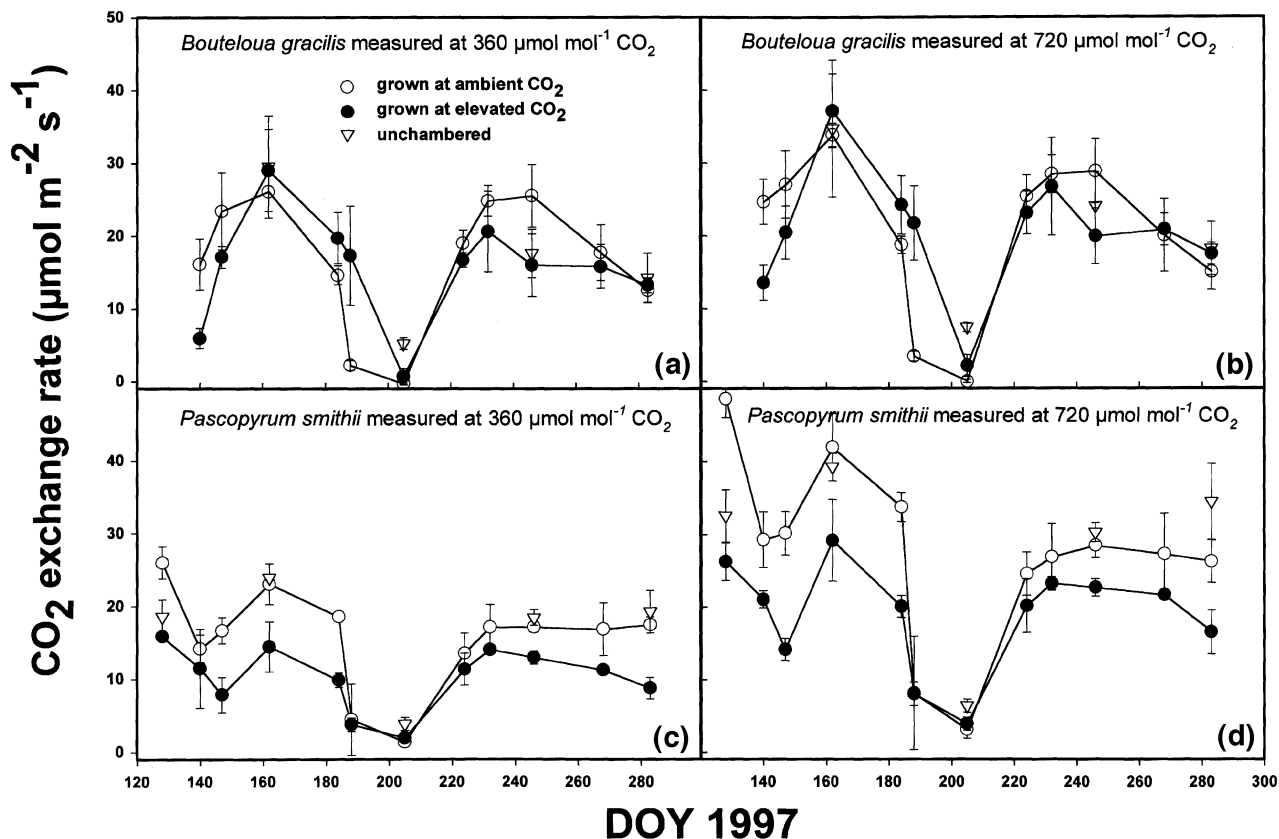


Fig. 6 Leaf CO₂ exchange rate of *Bouteloua gracilis* (a,b) and *Pascopyrum smithii* (c,d) at the open-top-chamber experiment in 1997. Ambient chambers had 360 ± 20 and elevated chambers had 720 ± 20 μmol mol⁻¹ CO₂ and unchambered were plots of equal ground area with no chamber on them. CO₂ exchange rate was measured at a leaf chamber concentration of either 360 or 720 μmol mol⁻¹ CO₂. Bars are standard errors.

due in part to higher than usual precipitation which resulted in sufficient mid-year soil water to support growth in the summer-defoliated quadrats. As a result, the potentially negative consequence of defoliation on removal of the photosynthetic surface was more than offset by positive factors including generation of a new, more efficient photosynthetic surface and improved water relations due to lowered leaf area. Under these conditions of relatively abundant late-season soil water supply, elevated CO₂ stimulated growth similarly in summer-defoliated quadrats as well as in quadrats which were not defoliated until the end of the growing season. In contrast, periods of above-average precipitation tended to eliminate the CO₂-induced growth responses in the tallgrass prairie, whether measured over a whole growing season, or just considering regrowth of summer-defoliated vegetation (Owensby *et al.* 1999).

The effect of elevated CO₂ on productivity in the shortgrass steppe and tallgrass prairie is primarily realized through improved water use efficiency (Hunt *et al.* 1996; Morgan *et al.* 1998; Owensby *et al.* 1999; discussion below), so responses are certain to be linked

to soil water. In tallgrass prairie where annual precipitation averages 840 mm (Owensby *et al.* 1999), higher-than-normal late season precipitation may improve water relations sufficiently to nullify any potential impact of elevated CO₂ on regrowth. Production on the semiarid shortgrass steppe, which receives an average of only 320 mm precipitation annually, is considerably more water-limited than the tallgrass prairie, so above-average precipitation late in the growing season is less likely to reduce the ability of elevated CO₂ to enhance regrowth of summer-defoliated vegetation. The contrasting results of these studies and grasslands point out the nature of CO₂ and defoliation responses is complex, involving interactions of the particular plant community with soil resources as well as timing and frequency of defoliation.

C₃/C₄ Responses. Despite predictions that plants possessing C₃ metabolisms are more responsive to increases in CO₂ above present ambient concentrations, we detected no significant effect of elevated CO₂ on the percentage composition of C₃ and C₄ grasses. However, in tallgrass prairie, Owensby *et al.* (1996) reported greater CO₂-responsiveness of the dominant C₄ grass, *Andropogon*

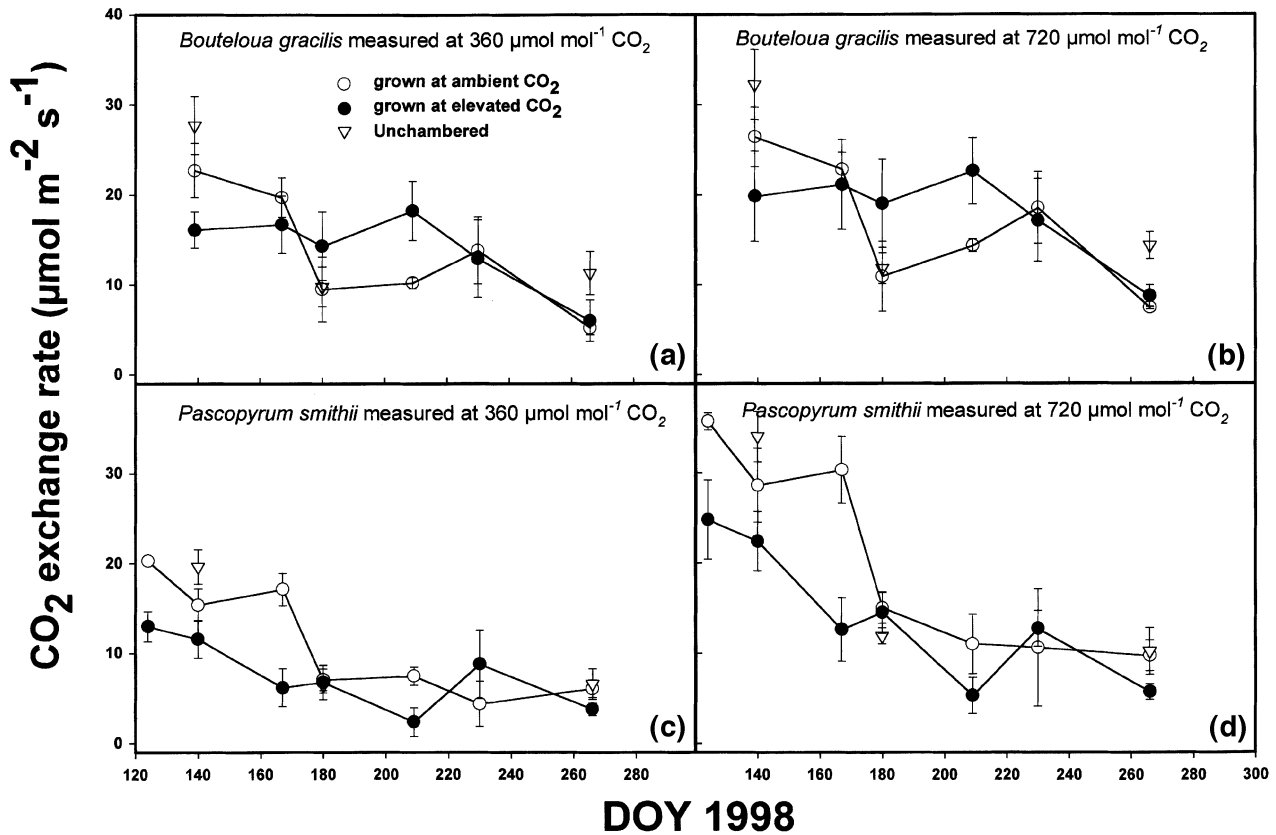


Fig. 7 Leaf CO₂ exchange rate of *Bouteloua gracilis* (A,B) and *Pascopyrum smithii* (C,D) at the open-top-chamber experiment in 1998. Ambient chambers had 360 ± 20 and elevated chambers had 720 ± 20 μmol mol⁻¹ CO₂ and unchambered were plots of equal ground area with no chamber on them. CO₂ exchange rate was measured at a leaf chamber concentration of either 360 or 720 μmol mol⁻¹ CO₂. Bars are standard errors.

gerardii compared to the C₃ grass, *Poa pratensis*, and suggested that the increased water use efficiency from CO₂ enrichment plus the lack of defoliation allowed the tall C₄ grass to out-compete the shorter C₃ grasses for light. Indeed, if defoliation had occurred in the tallgrass study, *P. pratensis* would likely have intercepted more radiation and experienced a stronger response to CO₂. In contrast, differences in height between photosynthetic classes are reversed in shortgrass steppe compared to tallgrass prairie; the dominant grass, *B. gracilis*, is C₄ and has a relatively prostrate growth habit compared to some of the important C₃ grasses like *P. smithii* and *S. comata*. Further, the effects of canopy architecture on species responses are unlikely to be very important in semiarid grasslands like the shortgrass steppe where leaf area index rarely exceeds one (Burke *et al.* 1998). Based on the growth responses for the first two years of this study plus results of independently grown shortgrass steppe grasses (Hunt *et al.* 1996), the intrinsic responses of C₃ and C₄ grasses of the shortgrass steppe to CO₂ enrichment appear similar.

Mechanisms underlying CO₂-induced growth enhancement. Although C₃ photosynthesis is limited more by present ambient CO₂ concentrations compared to C₄ photosynthesis, our results did not indicate that photosynthesis of *P. smithii* leaves grown and measured at 720 μmol mol⁻¹ was stimulated any more than in *B. gracilis* leaves. Reductions in photosynthesis of *P. smithii* leaves grown under elevated compared to ambient CO₂ concentrations when measured at the same CO₂ concentration indicate a down-ward type of photosynthetic acclimation. Photosynthetic acclimation in leaves of *P. smithii* grown at elevated CO₂ reduced the photosynthetic advantage of those leaves sufficiently that rates were often similar in ambient and elevated chambers. Acclimation was consistent and often substantial, and probably due to low N status of the vegetation (Morgan *et al.* 1994a; Sage 1994; Ghannoum & Conroy 1998). This may tend to limit the photosynthetic advantage for C₃ grasses in this ecosystem under elevated CO₂. Conversely, photosynthesis under elevated CO₂ was enhanced in *B. gracilis* leaves on several sampling dates, presumably due to improved

water relations, but also perhaps due to a direct stimulation of photosynthesis resulting from elevated CO₂ (LeCain & Morgan 1998; Morgan *et al.* 1994a). These results suggest that, for shortgrass steppe vegetation, separating plant growth responses to CO₂ based on photosynthetic class may not conform well to photosynthetic performance of these two groups determined in laboratories on well-watered and fertilized plants. Furthermore, they explain why for two years, we could detect no significant growth response difference between C₃ and C₄ grasses to CO₂ enrichment.

The consistently higher leaf water potentials of *B. gracilis*, *P. smithii* and *S. comata* under elevated CO₂ resulted from both improved soil water status and also to partial stomatal closure. These improved water relations can be important in a number of physiological functions besides photosynthesis. For instance, improved water relations under CO₂ enrichment can increase stand photosynthetic activity by enhancing leaf area expansion, without necessarily increasing specific photosynthetic activity (Gifford & Morrison 1985; Wand 1999). We suspect that in semiarid ecosystems like the shortgrass steppe, the benefit of these types of water relation responses will be as or more important than direct stimulations of photosynthesis, especially under conditions where downward photosynthetic acclimation occurs in dominant C₃ grasses like *P. smithii*.

While the short-term responses to this system are driven primarily by water relations, the soil N cycle may become an important factor determining its long-term responses. Despite previous growth chamber work that indicated reduced N concentrations in leaves of *P. smithii*, and to a lesser extent, *B. gracilis* when exposed to enriched CO₂ atmospheres (Read *et al.* 1997), this study provided only limited evidence for lower N concentrations in CO₂-enriched grass shoots. These limited findings are in agreement with other grassland studies which showed a decline in foliage N concentration under CO₂ enrichment (Owensby *et al.* 1993a; Hebeisen *et al.* 1997). If these responses become more pronounced with time, then in addition to water relations, the long-term responses of the shortgrass steppe will need to be re-evaluated in terms of the N cycle. However, while low system N tends to reduce CO₂-induced enhancements in productivity of fertile grasslands (Hebeisen *et al.* 1997; Zanetti *et al.* 1997), the relative impact of this on the shortgrass steppe will likely be less since water is such an important limiting factor in semiarid grasslands.

Global Change Implications: Our chambers had two important, but unintentional effects; warming and desiccation. The warming was in the order of 2.6 °C, the approximate warming expected to occur in the next hundred years. The desiccation which occurred simulates what will happen with warmer future

temperatures, assuming precipitation amounts remain similar: increased evapotranspiration, resulting in less available soil water. Despite the desiccating chamber environment, production was enhanced over the non-chambered controls. Plant growth inside the chambers commenced earlier in the year because of the warmer temperatures. This earlier start was probably responsible for the seasonal growth stimulation compared to non-chambered plants. Assuming these effects were the primary determinants of the chamber effects on plant response, our results suggest that in future CO₂-enriched and warmer climates, production on the shortgrass steppe should be enhanced significantly from that occurring now.

Conclusions

This study confirms what was observed in previous controlled-environment studies, that CO₂ enrichment will lead to substantial increases in above-ground productivity of the shortgrass steppe of northern Colorado, and that the growth responses are similar in dominant C₃ and C₄ grasses of this region. Although the photosynthetic mechanism of C₄ species is known to be nearly CO₂-saturated at present ambient CO₂ concentrations, while that of C₃ species is strongly CO₂-limited, we found no evidence that photosynthetic performance at elevated CO₂ was stimulated any more in the C₃ grass, *P. smithii*, compared to the dominant C₄ grass of this system, *B. gracilis*. We suspect that the primary effect of CO₂ enrichment on growth enhancement is due to improved water relations, and evidence to date suggests similar effects for both C₃ and C₄ grasses of the shortgrass steppe. We expect that CO₂-induced growth responses will be more consistent in the shortgrass steppe than in the subhumid tallgrass prairie. Despite an apparent desiccating environment in the chambers due to higher temperatures, productivity was enhanced in the chambers, with the highest production occurring under elevated CO₂. These data suggest increased productivity of the shortgrass steppe in future warmer, CO₂-enriched environments.

Acknowledgements

We would like to acknowledge the help of Doug Whitt, who helped design the chambers, Dean Ackerman, Barry Weaver, and Dennis Mueller who contributed to the design and built the chambers, Scott Andre, Mary Ashby, Jeff Thomas, Jim Nelson, Mary Smith, Susan Crookall, and Larry Tisue, for technical assistance, Clenton Owensby and Lisa Auen for technical chamber design advice, Gary Richardson for statistical advice, and Bill Parton and Dennis Ojima for help in designing the study.

We thank Justin Derner, Clenton Owensby and Jennifer King for helpful comments on the manuscript. This research was supported, in part, from funding received from the NSF-TECO award IBN-9524068, NSF award DEB-9708596, and from the NSF Shortgrass Steppe LTER Project DEB 9350273.

References

- Alcamo J, Kreileman GJJ, Bollen JC, et al. (1996) Baseline scenarios of global environmental change. *Global Environmental Change*, 6:261-303.
- Baxter R, Bell SA, Sparks TH, Ashenden TW, Farrar JF (1995) Effects of elevated CO₂ concentration on three montane grass species III. Source leaf metabolism and whole plant carbon partitioning. *Journal of Experimental Botany*, 46:917-929.
- Bowes G (1993) Facing the inevitable: plants and increasing atmospheric CO₂. *Annual Review of Plant Physiology and Plant Molecular Biology*, 44:309-332.
- Burke IC, Lauenroth WK, Vinton MA, Hook PB, Kelly RH, Epstein HE, Aguiar MR, Robles MD, Aguilera MO, Murphy KL, Gill RA (1998) Plant-soil interactions in temperate grasslands. *Biogeochemistry*, 42:121-143.
- Chiariello NR, Field CB (1996) Annual grassland responses to elevated CO₂ in multiyear community microcosms. In: *Carbon Dioxide, Populations, and Communities* (eds Korner C, Bazzaz FA), pp 139-157, Academic Press, Inc., San Diego, CA.
- Drake BG, González-Meler MA, Long SP (1996) More efficient plants: A consequence of rising atmospheric CO₂? *Ann. Rev. Plant Physiology and Molecular Biology*, 48:607-637.
- Ghannoum O, Conroy JP (1998) Nitrogen deficiency precludes a growth response to CO₂ enrichment in C-3 and C-4 Panicum grasses. *Australian Journal of Plant Physiology*, 25:627-636.
- Ghannoum O, von Caemmerer S, Barlow EWR, Conroy JP (1997) The effect of CO₂ enrichment and irradiance on the growth, morphology and gas exchange of a C₃ (*Panicum laxum*) and a C₄ (*Panicum antidotale*) grass. *Australian Journal of Plant Physiology*, 24:227-237.
- Ghannoum O, von Caemmerer S, Ziska LH, Conroy JP (2000) The growth response of C₄ plants to rising atmospheric CO₂ partial pressure: a reassessment. *Plant, Cell and Environment*, 23:931-942.
- Gifford RM, Morrison JIL (1985) Photosynthesis, water use and growth of a C₄ grass stand at high CO₂ concentration. *Photosynthesis Research*, 7: 77-90.
- Hebeisen T, Lüscher A, Zanetti S, Fischer BU, Hartwig UA, Frehner M, Hendry GR, Blum H, Nosberger J (1997) Growth response of *Trifolium repens* L. and *Lolium perenne* L. as monocultures and bi-species mixture to free air CO₂ enrichment and management. *Global Change Biology*, 3:149-160.
- Hunt HW, Elliott ET, Detling JK, Morgan JA, Chen, D-X (1996) Responses of a C₃ and C₄ perennial grass to elevated CO₂ and climate change. *Global Change Biology*, 2, 35-47.
- Kirkham MB, He H, Bolger TP, Lawlor DJ, Kanemasu ET (1991) Leaf photosynthesis and water use of big bluestem under elevated carbon dioxide. *Crop Science*, 31, 1589-1594.
- Klippel GE, Costello DF (1960) Vegetation and cattle response to different intensities of grazing on shortgrass ranges on the central Great Plains. *USDA Tech. Bull.* 1216, USDA, Washington, D.C.
- Koch GW, Mooney HA (1996) Response of terrestrial ecosystems to elevated CO₂: A synthesis and summary. In: *Carbon Dioxide and Terrestrial Ecosystems* (eds Korner C, Bazzaz FA), pp 415-429, Academic Press, Inc., San Diego, CA.
- Lauenroth WK, Milchunas DG (1991) Short-grass steppe. In: *Ecosystems of the World 8A: Natural Grasslands* (ed Coupland RT), pp 183-226, Elsevier, Amsterdam.
- LeCain DR, Morgan JA (1998) Growth, gas exchange, leaf nitrogen and carbohydrate concentrations in NAD-ME and NADP-ME C₄ grasses in elevated CO₂. *Physiologia Plantarum*, 102:297-306.
- Menke JW, Trlica MJ (1983) Effects of single and sequential defoliations on the carbohydrate reserves of four range species. *Journal of Range Management*. 36:70-74.
- Milchunas DG, Lauenroth WK (1993) A quantitative assessment of the effects of grazing on vegetation and soils over a global range of environments. *Ecological Monographs*, 63:327-366.
- Milchunas DG, Lauenroth WK, Sala OE (1988) A generalized model of the effects of grazing by large herbivores on grassland community structure. *American Naturalist* 132:87-106.
- Milchunas DG, Varnamkasti AS, Lauenroth WK, Goetz H (1995) Forage quality in relation to long-term grazing history, current-year defoliation, and water resource. *Oecologia*, 101:366-374.
- Mooney HA, Drake BG, Luxmoore RJ, Oechel WC, Pitelka LF (1991) Predicting ecosystem responses to elevated CO₂ concentrations. *BioScience*, 41, 96-104.
- Morgan JA, Hunt HW, Monz CA, LeCain DR (1994a) Consequences of growth at two carbon dioxide concentrations and two temperatures for leaf gas exchange in *Pascopyrum smithii* (C₃) and *Bouteloua gracilis* (C₄). *Plant, Cell and Environment* 17:1023-1033.
- Morgan JA, Knight WG, Dudley LM, Hunt HW (1994b) Enhanced root system C-sink activity, water relations and aspects of nutrient acquisition in mycotrophic *Bouteloua gracilis* subjected to CO₂ enrichment. *Plant and Soil*, 165: 139-146.
- Morgan JA, LeCain DR, Read JJ, Hunt HW, Knight WG (1998) Photosynthetic pathway and ontogeny affect water relations and the impact of CO₂ on *Bouteloua gracilis* (C₄) and *Pascopyrum smithii* (C₃). *Oecologia*, 114, 483-493.
- Nie D, He H, Mo G, Kirkham MB, Kanemasu ET (1992) Canopy photosynthesis and evapotranspiration of rangeland plants under doubled carbon dioxide in closed-top chambers. *Agricultural and Forest Meteorology*, 61, 205-217.
- Owensby CE, Coyne PI, and Auen LM (1993a) Nitrogen and phosphorus dynamics of a tallgrass prairie ecosystem exposed to elevated carbon dioxide. *Plant Cell and Environment* 16:843-850.
- Owensby CE, Coyne PI, Ham JM, Auen LM, Knapp AK (1993b) Biomass production in a tallgrass prairie ecosystem exposed to ambient and elevated CO₂. *Ecological Applications*, 3, 644-653.
- Owensby CE, Ham JM, Knapp A, Rice CW, Coyne PI, Auen LM (1996) Ecosystem-level responses of tallgrass prairie to elevated CO₂. In: *Carbon Dioxide and Terrestrial Ecosystems* (eds Korner C, Bazzaz FA), pp 147-162. Academic Press, Inc., San Diego, CA.
- Owensby CE, Ham JM, Knapp AK, Auen LM (1999) Biomass production and species composition change in a tallgrass

- prairie ecosystem after long-term exposure to elevated atmospheric CO₂. *Global Change Biology* 5, 497-506.
- Poorter H (1993) Interspecific variation in the growth response of plants to an elevated ambient CO₂ concentration. *Vegetatio*, 104/105, 77-97.
- Poorter H, Roumet C, Campbell BD (1996) Interspecific variation in the growth response of plants to elevated CO₂: a search for functional types. In: *Carbon Dioxide, Populations, and Communities* (eds Korner C, Bazzaz FA), pp 375-412. Academic Press, Inc., San Diego.
- Read JJ, Morgan JA, Chatterton NJ, Harrison PA (1997) Gas exchange and carbohydrate and nitrogen concentrations in leaves of *Paspopyrum smithii* (C₃) and *Bouteloua gracilis* (C₄) at different carbon dioxide concentrations and temperatures. *Annals of Botany* 79:197-206.
- Sage RF (1994) Acclimation of photosynthesis to increasing atmospheric CO₂: The gas exchange perspective. *Photosynthesis Research*, 39, 351-368.
- Sionit N, Patterson DT (1984) Responses of C₄ grasses to atmospheric CO₂ enrichment. I. Effect of irradiance. *Oecologia*, 65, 30-34.
- Stitt M (1991) Rising CO₂ levels and their potential significance for carbon flow in photosynthetic cells. *Plant, Cell and Environment*, 14, 311-317.
- Trlica MJ, Buwai M, Menke JW (1977) Effects of rest following defoliations on the recovery of several range species. *Journal of Range Management*, 30:21-27.
- Wand SJE (1999) Ecophysiological responses of *Themeda triandra* Forsk. and other southern African C₄ grass species to increases in atmospheric CO₂ concentrations. DS Thesis, University of Cape Town, South Africa.
- Wand SJE, Midgley GF, Jones MH, Curtis PS (1999) Responses of wild C₄ and C₃ grass (Poaceae) species to elevated atmospheric CO₂ concentration: a meta-analytic test of current theories and perceptions. *Global Change Biology* 5:723-741.
- Wilsey BJ, McNaughton SJ, Coleman JS (1994) Will increases in atmospheric CO₂ affect regrowth following grazing in C₄ grasses from tropical grasslands? A test with *Sporobolus kentrophyllus*. *Oecologia*, 99:141-144.
- Wilsey BJ, Coleman JS, McNaughton JS (1997) Effects of elevated CO₂ and defoliation on grasses: a comparative ecosystem approach. *Ecological Applications*, 7:844-853.
- Zanetti S, Hartwig UA, van Kessel C, Lüscher, Hebeisen T, Frehner M, Fischer BU, Hendrey GR, Blum H and Nösberger (1997) Does nitrogen nutrition restrict the CO₂ response of fertile grassland lacking legumes? *Oecologia* 112:17-25.
- Ziska LH, Bunce JA (1997) Influence of increasing carbon dioxide concentration on the photosynthetic and growth stimulation of selected C₄ crops and weeds. *Photosynthesis Research*, 54:199-208.