



## Elevated CO<sub>2</sub> increases soil moisture and enhances plant water relations in a long-term field study in semi-arid shortgrass steppe of Colorado

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Received 10 February 2003. Accepted in revised form 26 September 2003

**Key words:** elevated CO<sub>2</sub>, leaf water potential, shortgrass steppe, soil moisture, water-use efficiency

### Abstract

Increasing atmospheric CO<sub>2</sub> has potentially significant impacts on the dynamics of water use and conservation in semi-arid rangelands. In this study we used large (15.5 m<sup>2</sup>) open top chambers to investigate effects of twice ambient CO<sub>2</sub> concentration (720 μL L<sup>-1</sup>) on plant and soil water relations of semi-arid shortgrass steppe (SGS) of northeastern Colorado from 1997 to 2001. Seasonal average soil moisture throughout the soil profile (0–15, 15–45, 45–75, 75–105 cm) was increased under elevated CO<sub>2</sub> compared to ambient CO<sub>2</sub> for much of the study period. When averaged across years, the greatest relative increase (elevated vs. ambient) in soil moisture occurred in the 75–105 cm depth increment (16.4%). Averaged over the study period, leaf water potential (Ψ<sub>leaf</sub>) was enhanced 24–30% under elevated CO<sub>2</sub> in the major warm- and cool-season grass species of the SGS (*Bouteloua gracilis*, C<sub>4</sub>, 28.5%; *Pascopyrum smithii*, C<sub>3</sub>, 24.7%; *Stipa comata*, C<sub>3</sub>, 30.4%), and the degree of responsiveness in Ψ<sub>leaf</sub> to elevated CO<sub>2</sub> did not differ between C<sub>3</sub> and C<sub>4</sub> plant functional types, but did differ between C<sub>3</sub> species. Water-use efficiency (WUE; g aboveground biomass harvested/ kg water consumed) was 43% higher in elevated (6.10) than ambient (4.27) CO<sub>2</sub> plots over the study period. Results suggest that a future, elevated CO<sub>2</sub> environment may result not only in increased plant productivity due to improved WUE, but also lead to increased water drainage and deep soil moisture storage in this semi-arid grassland ecosystem. This, along with the ability of the major grass species to maintain a favorable water status under elevated CO<sub>2</sub>, should result in the SGS being less susceptible to prolonged periods of drought. However, increases in deep soil water may eventually favor deeper-rooted over shallow-rooted species.

### Introduction

Grasslands are highly sensitive to changes in water supply, with small changes in water balance often causing large changes in ecosystem structure and function (Sala et al., 1992). With atmospheric CO<sub>2</sub> concentrations projected to exceed 700 μL L<sup>-1</sup> by the end of this century (Mearns, 2000), determining how elevated CO<sub>2</sub> may affect soil moisture in semi-arid

environments will be crucial in predicting resulting grassland ecosystem structure, function and water use. Previous studies have reported increased soil moisture under elevated CO<sub>2</sub> in semi-arid C<sub>3</sub> annual grasslands in California (Fredeen et al., 1997), mesic, C<sub>3</sub>/C<sub>4</sub> perennial tallgrass prairie in Kansas (Owensby et al., 1993, 1999; Ham et al., 1995; Bremer et al., 1996), and mesic C<sub>3</sub> perennial grasslands in Switzerland (Niklaus et al., 1998) and Sweden (Sindhøj et al., 2000). How elevated CO<sub>2</sub> will affect the water balance in semi-arid rangeland with mixed C<sub>3</sub>/C<sub>4</sub> perennial ve-

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getation such as that found in the shortgrass steppe of North America is unknown.

Effects of elevated  $\text{CO}_2$  on water conservation at the leaf level are fairly well understood, with most studies demonstrating that in drier conditions under elevated  $\text{CO}_2$ , leaf water-use efficiency is improved (Morison, 1993; Owensby et al., 1993; Jackson et al., 1994). However, effects of elevated  $\text{CO}_2$  on ecosystem water balance needs more attention (Owensby et al., 1999). For conservation of water at the ecosystem-level to occur, the reduction in water loss per unit leaf area under elevated  $\text{CO}_2$  must not be offset by an increase in total evaporative leaf surface (Field et al., 1997; Niklaus et al., 1998; Volk et al., 2000) and reduced stand evapotranspiration (ET) must be evident (Drake et al., 1997). Direct observations of reduced ET (Nie et al., 1992; Ham et al., 1995; Bremer et al., 1996; Fredeen et al., 1997; Field et al., 1997) and increased soil moisture under elevated  $\text{CO}_2$  are commonly observed in grassland ecosystems (Field et al., 1995) where availability of water is often limiting and effects of stomatal closure on transpiration are not negated by an increase in leaf area (Polley et al., 1997; Lockwood, 1999).

Decreased stomatal conductance ( $g_s$ ) under elevated  $\text{CO}_2$  is often associated with increased leaf water potential ( $\Psi_{\text{leaf}}$ ) which reflects improved plant water status and increased drought tolerance (Tyree and Alexander, 1993). Mechanisms that contribute to improved  $\Psi_{\text{leaf}}$  under elevated  $\text{CO}_2$  are not fully known but have been attributed to reduced  $g_s$  and increased plant hydraulic conductance along with improved leaf turgor associated with osmotic adjustment (Tyree and Alexander, 1993; Wullschleger et al., 2002). There appears to be little difference in stomatal sensitivity to elevated  $\text{CO}_2$  between  $\text{C}_3$  and  $\text{C}_4$  species (Morison and Gifford, 1983; Polley et al., 1997; Wand et al., 1999). However, direct comparisons of the degree to which  $\Psi_{\text{leaf}}$  are altered by elevated  $\text{CO}_2$  among naturally co-occurring  $\text{C}_3$  and  $\text{C}_4$  grass species are rare. Further, despite knowledge that different rooting patterns of co-occurring natives are beneficial for maximizing the efficiency of soil resource utilization (Weaver and Albertson, 1956), the affect of such rooting patterns on species  $\Psi_{\text{leaf}}$  responses to elevated  $\text{CO}_2$  are unknown.

Water is the most important factor that governs ecosystem structure and function in the semi-arid shortgrass steppe (SGS) of North America (Sala et al., 1992). Large temporal variability in rainfall and the preponderance of small precipitation events in this

region increase the importance of soil water stored below the evaporative zone for the perpetuation and stability of perennial shortgrass vegetation (Singh et al., 1998). Any substantial increase in soil moisture under elevated  $\text{CO}_2$  or an increase in the percolation of precipitation deeper into the soil profile (Grünzweig and Körner, 2001) could have a considerable impact on the production and survival of SGS species during prolonged periods of drought.

In this study, we used large ( $15.5 \text{ m}^2$ ) open top chambers to examine the effects of elevated  $\text{CO}_2$  on plant water relations, ecosystem water use efficiency, soil moisture dynamics and root distributions of dominant  $\text{C}_3$  (*Pascopyrum smithii* and *Stipa comata*) and  $\text{C}_4$  (*Bouteloua gracilis*) grass species in the SGS of northeastern Colorado. Specifically, we tested the hypotheses that (1) elevated  $\text{CO}_2$  will increase soil water content, especially in lower soil depths that are free from evaporative demand, (2) elevated  $\text{CO}_2$  will increase  $\Psi_{\text{leaf}}$  in three important grass species of the SGS, (3) the degree of responsiveness in  $\Psi_{\text{leaf}}$  to elevated  $\text{CO}_2$  will be the same between  $\text{C}_3$  and  $\text{C}_4$  grass species, and (4) water-use efficiency (g of above-ground biomass harvested/kg of water consumed during the growing season) will be greater under elevated  $\text{CO}_2$ .

## Methods

### Study site

Experiments were conducted at the USDA-ARS Central Plains Experimental Range (CPER) located in the northern portion of the shortgrass steppe (SGS) about 60 km northeast of Fort Collins, Colorado ( $40^\circ 49' \text{ N}$ ,  $107^\circ 47' \text{ W}$ ; elevation 1650 m). Mean annual precipitation averaged 320 mm over the past 55 years with about 80% occurring between April and September (Lauenroth and Milchunas, 1991). Long-term monthly average air temperatures range from minus  $5^\circ \text{ C}$  in January to  $22^\circ \text{ C}$  in July. The study site is located about 20 km south of the continental transition between  $\text{C}_3$  and  $\text{C}_4$  dominance (Epstein et al., 1997). Vegetation is dominated by *Bouteloua gracilis* (H.B.K.) Lag. (blue grama) a  $\text{C}_4$  grass, but  $\text{C}_3$  grasses *Pascopyrum smithii* (Rydb.) A. Love (western wheatgrass) and *Stipa comata* Trin and Rupr. (needle and thread) are also a major vegetation component. Soil at the experimental site is a Remmit fine sandy loam (Ustollic camborthids). Sand content ranges from approximately 80% at the surface to 65% at 60–75 cm,

while clay content is 13% at the surface and 17–20% at 60–75 cm.

#### *CO<sub>2</sub> treatment and chamber environment*

Large open top chambers (4.5 m diameter, approximately 3.8 m height, enclosing 15.5 m<sup>2</sup> ground area, or 60.5 m<sup>3</sup> volume) were used to investigate effects of elevated CO<sub>2</sub> on native vegetation of the SGS. The experiment was established during the spring of 1997 on a six ha field of native rangeland with a mixture of cool- and warm-season grass species. The field was divided into three blocks based on uniformity of vegetation; three 15.5 m<sup>2</sup> circular plots were randomly chosen as experimental plots within each block. Open top chambers were placed on two plots in each of the three blocks (six total) from late March until mid-October of each year. Within each block, one chamber was randomly assigned an ambient CO<sub>2</sub> treatment ( $\sim 360 \mu\text{L L}^{-1}$ ), the other an elevated CO<sub>2</sub> treatment ( $\sim 720 \mu\text{L L}^{-1}$ ). Each block also had an unchambered plot of equal ground area, which was used to monitor effects of the chambers. See Morgan et al., (2001) for a detailed description of experimental and open top chamber design.

Elevated CO<sub>2</sub> chambers were maintained at a concentration of  $720 \pm 15 \mu\text{L L}^{-1}$  by injecting 100% CO<sub>2</sub> into input fans where it was mixed with ambient air before being delivered into chambers. CO<sub>2</sub> concentration inside chambers was monitored regularly by drawing air from a sampling manifold, a 1.5 m long by 5 cm diameter perforated PVC tube positioned 2 m high across the center of chambers, to an infrared gas analyzer (LI-COR LI6262; LI-COR, Lincoln, NE, USA). Soil within chambers was isolated by an aluminum flange buried 0.8 m deep around the outside edge of chamber walls. Openings in the top of each chamber were reduced to 0.75 m diameter to allow better control of chamber CO<sub>2</sub> and also to facilitate the measurement of steady state chamber gas fluxes (not reported here). Incident precipitation upon the chamber tops was channeled into reservoirs and immediately pumped onto chambered plots using an automated sprinkler system. The precipitation catchment system was not 100% efficient, so the amount of applied water was carefully monitored with flow meters and compared to a rain gage on site to determine deficiencies for each precipitation event. Deficiencies were generally small (<10%) and were made up at least weekly by applying water early in the morning via the automated sprinkler system. Consequently,

chambered plots (ambient and elevated CO<sub>2</sub>) and unchambered controls received the same total amount of precipitation.

Detailed environmental parameters were measured on all plots and sampled with a computer-based data acquisition system (Keithley Metrabyte WORKHORSE; Keithley Instruments Inc., Taunton, MA). Measurements of microclimate were made each minute, and hourly averages of air and soil temperature, and 15 minute averages of photosynthetically active radiation (PAR) were recorded. Air temperature was measured at a height of 15 cm using thin-wire thermocouples and soil temperature was measured at a depth of 10 cm using heavy shielded thermocouple wire. Photosynthetically active radiation was measured above the plant canopy using a point quantum sensor (LI-COR LI-190SA).

#### *Soil moisture and leaf water potential*

Volumetric soil moisture content was measured from an access tube within each plot on a weekly basis using a Troxler model 4301 neutron probe (Troxler Electronics Lab., Research Triangle Park, NC, USA) which had been calibrated against soil at the experimental site. Time Domain Reflectometry (TDR) was also used weekly to measure soil moisture in the top 15 cm of the soil profile where neutron scattering (under varying moisture conditions) may reduce accuracy of neutron probe measurements. Depth increments for soil moisture analyses were 0–15 (TDR), 15–45, 45–75 and 75–105 cm (neutron probe placed in center of each depth increment).

Leaf water potential ( $\Psi_{\text{leaf}}$ ) was measured weekly throughout each growing season (April–October) on 1–2 leaves each of *B. gracilis*, *P. smithii* and *S. comata* in all plots with a Scholander-type pressure chamber (PMS Instrument Company, Corvallis, OR, USA). Measurements were taken mid-morning (1000–1145 hrs MST) when plants were typically most active and sampled leaves were processed immediately after being cut.

#### *Water-use efficiency*

Aboveground plant biomass harvests were conducted in the southern half of all chambers at two different times during each growing season. Vegetation was clipped to crown-level from half of the harvest area (1.73 m<sup>2</sup> area) in late July, which coincides with peak standing crop. This defoliation protocol, which removed half of the standing vegetation, was done

to simulate defoliation by cattle under recommended stocking rates, which removes slightly less than half of the summer standing crop (Morgan et al., in press). A second aboveground biomass harvest was conducted after plant senescence in late October, involved the entire sample area (3.46 m<sup>2</sup> total area), and included re-growth of the summer-defoliated harvest area, plus defoliation of the other half of the sample area that had not been clipped at the summer harvest. Total seasonal aboveground biomass was the sum of all harvested biomass. Water-use efficiency was calculated as the total aboveground biomass (g) harvested per total amount of water (kg) lost from the same area during the same time period. Total water lost was calculated by subtracting season-beginning soil water content from the total of season-ending plus precipitation. We assumed there was minimal run-off and that percolation of water rarely reached below 105 cm (deepest neutron probe reading) because of coarse-textured surface soils and preponderance of small rainfall events.

#### *Root biomass distribution of dominant grasses*

After termination of the CO<sub>2</sub> treatments, chambers were removed from the plots, and 100 cm wide, 20 cm thick, and 75 cm deep (150 L volume) soil monoliths were extracted for root studies and soil washed away at 0–20, 20–45 and 45–75 depth intervals. Once cleaned of soil, roots were separated by species by gently un-tangling the roots that were attached to crowns. Root separation was facilitated with a commercial hair detangler solution (main ingredients: cetearlyl alcohol, cyclomethicone and polysorbate 20). Roots were cut from the crowns, by depth increment, washed and cleaned of litter, dried at 60 °C, and weighed. In this report we show the root data from only the site dominant species, *Bouteloua gracilis*, *Pascopyrum smithii* and *Stipa comata*.

#### *Statistical analyses*

The fixed effects of CO<sub>2</sub> treatment (includes non-chambered control, ambient chamber and elevated chamber) on seasonal soil water content by depth, leaf water potential ( $\Psi_{\text{leaf}}$ ), species  $\Psi_{\text{leaf}}$  responsiveness and water-use efficiency were analyzed using the SAS PROC MIXED analysis (SAS Institute Inc., Cary, NC, USA), with year represented as a repeated measure and block designated as a random effect. Means comparisons were made using Least Squares Means (Tukey's HSD *P*-value adjustment) with differences

reported as significant when  $P < 0.05$ . Monoliths were collected only in year 2002, therefore there was no 'year' effect, and the root data were analyzed using SAS PROC ANOVA. Differences between species were analyzed with the LSD means comparison test.

## **Results**

### *Seasonal climate and chamber environment*

Growing season precipitation ranged from 247 mm in 2000 to 523 mm in 1999, compared to the long-term average of 280 mm. Both 1997 (480 mm) and 1999 (523 mm) were well above average rainfall years, 1998 (302 mm) and 2001 (311 mm) were near average and 2000 (247 mm) was a dry year. Although the precipitation total in 2001 was near the long-term average, spring rains failed to recharge the soil profile following a dry year (2000) and a summer drought decreased soil moisture values to the lowest recorded during the study (Figure 1).

Air temperature was on average 2.6 °C higher inside the chambers than outside, and soil temperature averaged 1.25 °C higher in the chambers. Lexan walls of the chambers caused a 6–8% reduction in PAR. However, daily PAR was reduced about 28% inside the chambers compared to the outside environment, with the majority of this reduction attributed to shading from the chamber framework.

### *Soil water content*

Analysis of variance results for the lowest three soil depth increments (15–45, 45–75, 75–105 cm measured with a neutron probe) showed significant ( $P < 0.05$ ) CO<sub>2</sub> treatment and year effects on volumetric soil water content, but no year by treatment interaction. Means separation of the treatment effects at these depths indicated that volumetric soil water content in the 15–45 and 75–105 cm depth increments was higher ( $P < 0.05$ ) under elevated CO<sub>2</sub> compared to ambient CO<sub>2</sub> over the course of the study period, with a strong trend ( $P = 0.08$ ) for higher soil water content at 45–75 cm (Figure 2). Soil water content in the 15–45 cm depth increment was higher in unchambered controls than both chambered ambient and elevated CO<sub>2</sub> plots over the course of the study. At 45–75 cm, soil moisture was similar between unchambered controls and chambered elevated CO<sub>2</sub> plots, both of which were higher than ambient CO<sub>2</sub> plots. Treatment comparisons for the 75–105 cm depth increment indicated

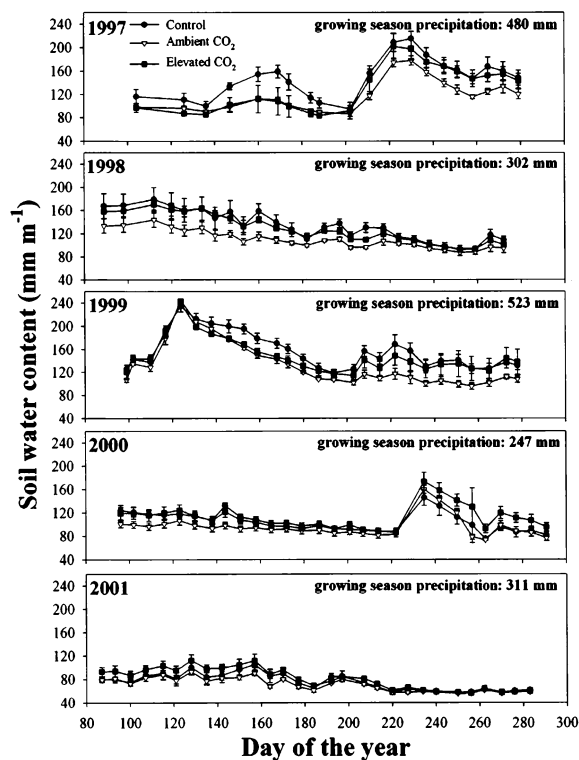


Figure 1. Total soil water content (mm) in the upper 1 m of the soil profile in unchambered controls and chambered ambient ( $360 \mu\text{mol mol}^{-1}$ ) and elevated ( $720 \mu\text{mol mol}^{-1}$ )  $\text{CO}_2$  plots in five growing seasons (1997–2001) on the shortgrass steppe of Colorado. Total growing season precipitation (mm) is noted for each year of the study.

that elevated  $\text{CO}_2$  plots had higher soil water content than both unchambered controls and ambient  $\text{CO}_2$  plots over the course of the study. The significant year effect at the three lower depths indicated that soil water content was generally highest in 1997–1999, and then declined in 2000 and 2001 (Figure 3).

A significant ( $P < 0.05$ ) treatment by year interaction was detected at the 0–15 cm depth increment, so treatment means comparisons in the upper soil layer were conducted among treatments for each study year. The results indicated that soil water content measured under elevated  $\text{CO}_2$  was higher than ambient  $\text{CO}_2$  in 1999 only (Figure 4). Soil water content in control plots was similar to elevated  $\text{CO}_2$  plots in all years except 1997, and was higher in controls than ambient  $\text{CO}_2$  plots in the first three years of the study (Figure 4).

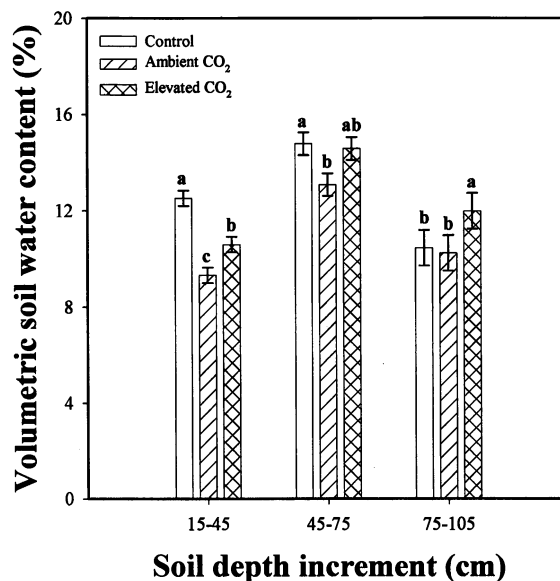


Figure 2. Percent volumetric soil water content ( $\pm 1$  S.E.;  $n = 3$ ) averaged over five years of study (1997–2001) of the 15–45 cm, 45–75 cm and 75–105 cm depth increments in unchambered control and chambered ambient ( $360 \mu\text{mol mol}^{-1}$ ) and elevated ( $720 \mu\text{mol mol}^{-1}$ )  $\text{CO}_2$  plots on the shortgrass steppe of Colorado. Different letters denote significant depth treatment effects ( $P < 0.05$ ; Least Square Means) at each soil depth.

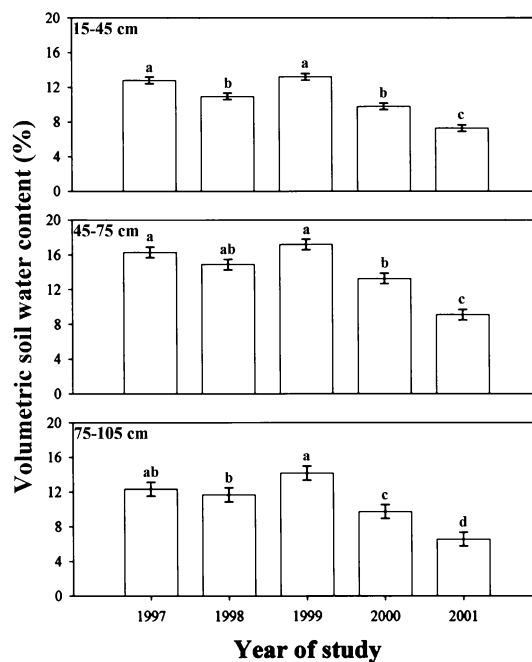


Figure 3. Percent volumetric soil water content ( $\pm 1$  S.E.;  $n = 3$ ) averaged across  $\text{CO}_2$  treatment (unchambered control, chambered ambient and elevated) of the 15–45 cm, 45–75 cm and 75–105 cm depth increments for each of the 5 years of study (1997–2001) on the shortgrass steppe of Colorado. Different letters denote significant year effects ( $P < 0.05$ ; Least Square Means) at each soil depth.

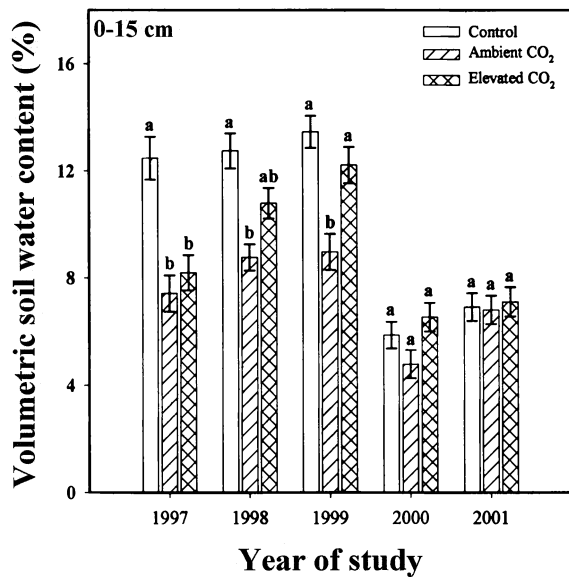


Figure 4. Seasonal average (1997–2001) percent volumetric soil water content ( $\pm 1$  S.E.;  $n=3$ ) of the 0–15 cm depth increment in unchambered control and chambered ambient ( $360 \mu\text{mol mol}^{-1}$ ) and elevated ( $720 \mu\text{mol mol}^{-1}$ )  $\text{CO}_2$  plots on the shortgrass steppe of Colorado. Different letters denote significant treatment effects ( $P < 0.05$ ; Least Square Means) within each year as called for by a significant treatment by year interaction.

#### Midday leaf water potential

Analysis of variance results indicated significant ( $P < 0.05$ ) treatment responses for midday leaf water potential ( $\Psi_{\text{leaf}}$ ) as well as significant ( $P < 0.05$ ) treatment by year interactions for the three common perennial grass species of the SGS. Treatment means comparisons conducted within each study year revealed that *Stipa comata* ( $C_3$ ) had higher  $\Psi_{\text{leaf}}$  under elevated  $\text{CO}_2$  for all five years of the study while *Pascopyrum smithii* ( $C_3$ ) and *Bouteloua gracilis* ( $C_4$ ) had higher seasonal  $\Psi_{\text{leaf}}$  in all but the first year of the study (Figure 5). Seasonal midday  $\Psi_{\text{leaf}}$  did not differ between ambient  $\text{CO}_2$  and unchambered control plots.

The degree of responsiveness of  $\Psi_{\text{leaf}}$  to elevated  $\text{CO}_2$ , or the percent increase in  $\Psi_{\text{leaf}}$  in elevated  $\text{CO}_2$  versus ambient  $\text{CO}_2$  plants, indicated significant species ( $P = 0.02$ ) and year ( $P < 0.0001$ ) effects (Table 1). *Stipa comata* was more responsive to elevated  $\text{CO}_2$  than its  $C_3$  counterpart *P. smithii*. However, neither of the  $C_3$  species differed from the  $C_4$  grass *B. gracilis* in their  $\Psi_{\text{leaf}}$  responsiveness to elevated  $\text{CO}_2$ . Averaged across species, the responsiveness of  $\Psi_{\text{leaf}}$  to elevated  $\text{CO}_2$  was higher in 1998 than the remaining four years.

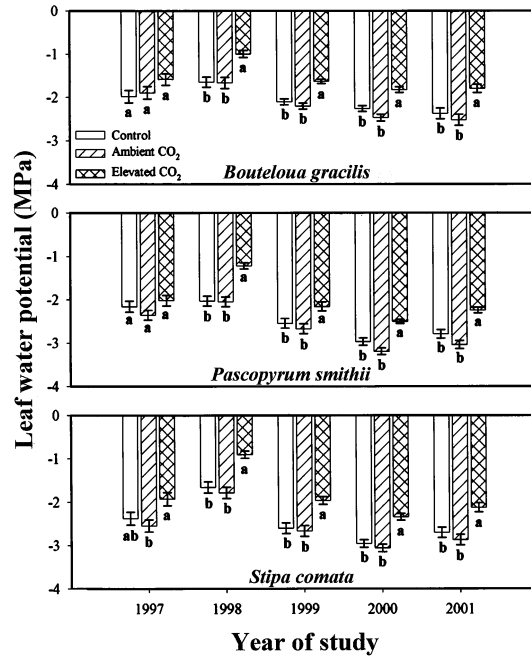


Figure 5. Seasonal average (1997–2001) midday (1000–1200 hrs MST) leaf water potential ( $\pm 1$  S.E.;  $n=3$ ) for three common grass species of the shortgrass steppe of Colorado in unchambered control and chambered ambient ( $360 \mu\text{mol mol}^{-1}$ ) and elevated ( $720 \mu\text{mol mol}^{-1}$ )  $\text{CO}_2$  plots. Different letters denote significant treatment effects ( $P < 0.05$ ; Least Square Means) within a year for each grass species.

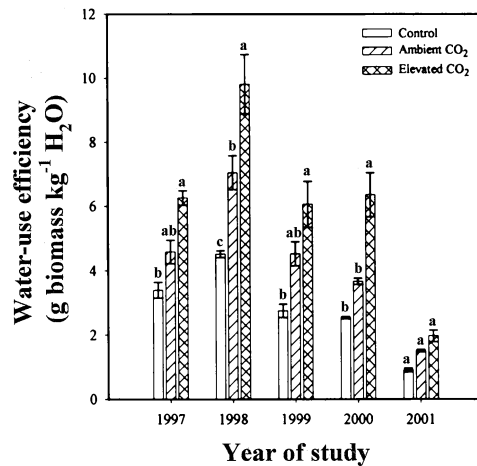


Figure 6. Yearly (1997–2001) water-use efficiency (g aboveground biomass harvested  $\text{kg}^{-1}$  water consumed;  $\pm 1$  S.E.;  $n=3$ ) for unchambered control and chambered ambient ( $360 \mu\text{mol mol}^{-1}$ ) and elevated ( $720 \mu\text{mol mol}^{-1}$ )  $\text{CO}_2$  plots in the shortgrass steppe of Colorado. Different letters denote significant treatment effects ( $P < 0.05$ ; Least Square Means) within a year.

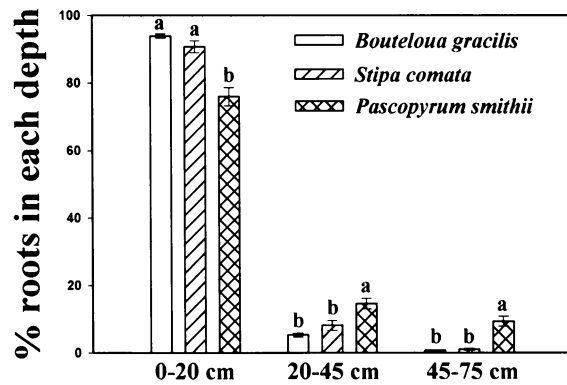
**Table 1.** The degree of responsiveness in leaf water potential ( $\Psi_{\text{leaf}}$ ) of three common short-grass steppe grass species (*Bouteloua gracilis*, *Pascopyrum smithii*, *Stipa comata*) to the CO<sub>2</sub> treatment (% increase in leaf water potential in elevated vs. ambient plants) over five years of CO<sub>2</sub> enrichment on the shortgrass steppe of Colorado. Different letters denote significant differences between means ( $P < 0.05$ ; Least Square Means) for species and year

Species	$\Psi_{\text{leaf}}$ Responsiveness (%)
<i>Bouteloua gracilis</i>	28.5 (ab)
<i>Pascopyrum smithii</i>	24.7 (b)
<i>Stipa comata</i>	30.4 (a)
Pr>F	0.0208
Year	
1997	22.7 (b)
1998	42.1 (a)
1999	23.8 (b)
2000	23.8 (b)
2001	26.9 (b)
Pr>F	<0.0001
Species*Year	
Pr>F	<0.4982

#### Water-use efficiency

Water-use efficiency of the harvested aboveground biomass was increased under elevated CO<sub>2</sub> compared to ambient CO<sub>2</sub> in 1998 and 2000, with strong trends for increased WUE in the remaining three years (Figure 6). Over the five years, WUE averaged 6.10 g kg<sup>-1</sup> under elevated CO<sub>2</sub> and was higher than the 4.27 g kg<sup>-1</sup> observed in ambient CO<sub>2</sub> plots. Control plots exhibited the lowest WUE across years at 2.82 g kg<sup>-1</sup>.

Total amount of water consumed over the course of each growing season differed very little between treatments (data not shown). Differences in WUE between treatments resulted from large differences in aboveground biomass. Over the five-year study, aboveground biomass harvested in elevated plots averaged 187 g m<sup>-2</sup>, 41% greater than the 133 g m<sup>-2</sup> harvested in ambient CO<sub>2</sub> chambered plots (Morgan et al., in press). The greatest increase in WUE (74%) with elevated CO<sub>2</sub> was observed in the drought year of 2000. This was also the year with the largest relative treatment differences in aboveground biomass, with 128 g m<sup>-2</sup> harvested in elevated plots compared



**Figure 7.** Root biomass distribution of three major grass species (*Bouteloua gracilis*, *Pascopyrum smithii* and *Stipa comata*) of the shortgrass steppe of Colorado. Panels depict the percentage of root biomass of each species found at three depths (0–20 cm, 20–45 cm and 45–75 cm) in monoliths extracted from unchambered control and chambered ambient (360  $\mu\text{mol mol}^{-1}$ ) and elevated (720  $\mu\text{mol mol}^{-1}$ ) CO<sub>2</sub> plots after 5 years of study. Data are averaged across CO<sub>2</sub> treatment.

to 76.8 g m<sup>-2</sup> in ambient plots, a 67% CO<sub>2</sub>-induced biomass increase.

#### Species distribution of roots by depth

There was no significant effect of CO<sub>2</sub> treatment, or chamber, on species' root weights determined at the end of the study, so data were averaged across treatments. Total dry weights of roots in the 75-cm deep soil monoliths differed by species, with 110 g m<sup>-2</sup> root biomass determined for *B. gracilis*, which was significantly ( $P < 0.05$ ) more than the 30 and 20 g m<sup>-2</sup> detected for *P. smithii* and *S. comata* roots, respectively. The distribution of roots at different depths differed among the species, with more than 99% of *B. gracilis* and *S. comata* roots occurring in the top 0–20 and 20–45 cm depth increments, while more than 10% of *P. smithii* roots were recovered in the lower 45–75 cm soil depth layer (Figure 7).

#### Discussion

Occupying the western edge of the central Great Plains of North America, the shortgrass steppe (SGS) is an ecosystem governed by water, and the response of this system to future increased atmospheric CO<sub>2</sub> concentrations will be largely through altered soil-plant water dynamics. Potential evaporation always exceeds precipitation in this semi-arid environment (Lauenroth and Milchunas, 1991, Sala et al., 1992) and water

stored below the evaporative zone (top 30 cm; personnel communication, Laj Ahuja) is extremely important in stabilizing this plant community during prolonged periods of drought (Singh et al., 1998). Over the course of this study we found that soil moisture under elevated CO<sub>2</sub> was increased by 11–16% in soil depth increments at (15–45 cm) or below (45–75, 75–105 cm) the evaporative zone. These increases are striking considering they occurred over a relatively long time frame which included extended drought periods when soil water content between elevated CO<sub>2</sub> and ambient CO<sub>2</sub> plots did not differ because little available water remained in the soil profile (Figure 1). Also, soil moisture below the evaporative zone under elevated CO<sub>2</sub> was similar to that in the unchambered controls (Figure 2) even though the elevated CO<sub>2</sub> plots were on average 2.6 °C warmer and supported 120% more aboveground biomass (Morgan et al., in press).

Elevated CO<sub>2</sub> increased soil moisture in the uppermost soil (0–15 cm) depth increment only in a very wet year (1999). High evaporative demand in this surface soil layer may have offset any direct effect of reduced plant transpiration on soil moisture under CO<sub>2</sub> enrichment, even though stable isotope partitioning of ET in the SGS has shown that the evaporation component of ET is significantly lower under elevated CO<sub>2</sub> (Ferretti et al., 2003). Increased soil moisture under elevated CO<sub>2</sub> at the deepest soil depth suggests that water percolated deeper into the soil profile and that less moisture was lost to evapotranspiration under elevated CO<sub>2</sub>. These results support our hypothesis that the relative increase in soil moisture under elevated CO<sub>2</sub> would be greatest at the lower soil depths where water can be stored free from evaporative demand and in a less densely rooted area. While some reports have suggested that increased soil drainage under elevated CO<sub>2</sub> has a negative effect on water balance in shallow soils (Grünzweig and Körner, 2001), this phenomenon enhances water storage in the deep fine sandy loam soils underlying large portions of the SGS.

On average, 67% of production in the SGS is belowground (Milchunas and Lauenroth, 2001), and competition for water is a major key to survival. Rooting patterns differed among the three major grass species at our study site (*B. gracilis*, *P. smithii*, *S. comata*; comprise approximately 88% of total aboveground biomass), with only *P. smithii* having a significant portion of roots in the 45–75 cm depth layer. Weaver and Albertson (1956) reported similar results for these species, although an intermediate rooting pattern has sometimes been observed for *S. comata*, with roots

extending deeper in the soil profile than *B. gracilis*, but not as deep as *P. smithii*. These types of differential rooting patterns are thought to reduce competition for water and increase coexistence among these species. However, species with deeper root systems, like *P. smithii*, may ultimately benefit more from deep soil moisture storage under elevated CO<sub>2</sub> than the shallow rooted *B. gracilis*.

We hypothesized that  $\Psi_{\text{leaf}}$  would be enhanced in all three of our study species due to CO<sub>2</sub>-induced increases in soil water content. Indeed, seasonal  $\Psi_{\text{leaf}}$  was higher in *B. gracilis* and *P. smithii* in 4 out of the 5 years, and higher in all 5 years in *S. comata*. Further,  $\Psi_{\text{leaf}}$  of *S. comata* was more responsive to changes in CO<sub>2</sub> than *P. smithii*. It seems plausible that  $\Psi_{\text{leaf}}$  of *P. smithii*, which has a deeper and more evenly depth-distributed root system, would have a more steady supply of soil water, and would be less sensitive to CO<sub>2</sub>-induced changes in soil water content than *S. comata*. Regardless of the mechanism, it does indicate that *S. comata* has the ability to increase plant water status to a greater degree under elevated CO<sub>2</sub> over a wide range of soil moisture contents. This may possibly lead to increased leaf turgor and allow *S. comata* to continue growth further into periods of drought. Indeed, of these three grass species, *S. comata* was the only one to have a significant aboveground productivity response to elevated CO<sub>2</sub> over the course of this study (Morgan et al., in press). However, the CO<sub>2</sub>-induced growth response of *S. comata* was supported in large part by enhanced seedling recruitment (Morgan et al., in press).

The response of  $\Psi_{\text{leaf}}$  in *B. gracilis* to elevated CO<sub>2</sub> did not differ from the two C<sub>3</sub> grasses. Previous reports have asserted that there is no significant difference in stomatal behavior of C<sub>3</sub> and C<sub>4</sub> species to elevated CO<sub>2</sub> (Morison and Gifford, 1983; Morison, 1985; Polley et al., 1997; Wand et al., 1999). Extensive leaf gas exchange measurements performed throughout this same field study (LeCain et al., 2003) confirmed this notion, as no significant differences in stomatal response to elevated CO<sub>2</sub> were observed between *B. gracilis* and *P. smithii*. Thus, we found no support for any differences in stomatal behavior between C<sub>3</sub> and C<sub>4</sub> plants that would differentially affect their leaf water relations responses to CO<sub>2</sub> enrichment.

In dry grasslands, enhanced aboveground production under elevated CO<sub>2</sub> has been attributed primarily to improved water relations (Owensby et al., 1993; Jackson et al., 1994; Chiariello and Field, 1996; Mor-



gan et al., 2001). In this study, soil moisture increased under elevated CO<sub>2</sub> (Figure 2), treatment differences ( $P < 0.05$ ) occurred in soil water content at the end of three (1997, 1999, 2000) out of five growing seasons (Figure 1), and consistently higher  $\Psi_{\text{leaf}}$  was observed under elevated CO<sub>2</sub> throughout the study (Figure 5). Averaged over all five years, weekly  $\Psi_{\text{leaf}}$  of the dominant C<sub>4</sub> grass (*B. gracilis*) and two dominant C<sub>3</sub> grasses (*P. smithii* and *S. comata*) increased 24–30% under elevated CO<sub>2</sub>. These results are similar to studies involving other C<sub>3</sub> and C<sub>4</sub> grass species (Owensby et al., 1993; Jackson et al., 1994). Leaf water potential in all three species was enhanced throughout each growing season, regardless of differences in soil moisture between ambient and elevated CO<sub>2</sub> plots. Possible mechanisms contributing to increased  $\Psi_{\text{leaf}}$  under elevated CO<sub>2</sub> may include decreased  $g_s$  and increased plant hydraulic conductance (Tyree and Alexander, 1993; Wullschleger et al., 2002). Stomatal conductance of *B. gracilis* and *P. smithii* leaves was reduced by 27–36% and leaf transpiration efficiency was increased by 75–80% under elevated CO<sub>2</sub> in the SGS (LeCain et al., 2003).

Elevated CO<sub>2</sub> increased WUE of harvested above-ground biomass from 31–74% compared to ambient CO<sub>2</sub>. This result concurs with those in a recent review (Wullschleger et al., 2002) that found most studies reported a 30–50% increase in WUE with elevated CO<sub>2</sub>. Whole-plant WUE ranged from no effect to a 180% increase under elevated CO<sub>2</sub> over a wide range of ecosystems and individual plant species (Wullschleger et al., 2002). In general, WUE appears affected more by elevated CO<sub>2</sub> under drought conditions (i.e., Field et al., 1997; Arp et al., 1998), which is supported by our results in 2000. However, the smallest increase in WUE due to CO<sub>2</sub> enrichment was measured in 2001 which, while having slightly above average precipitation, had the lowest recorded soil moisture during this study. The highly variable timing of precipitation in the SGS (Lauenroth and Milchunas, 1991; Sala et al., 1992) may be an important factor in plant productivity and water relations responses of this system to elevated CO<sub>2</sub>. In our study, the water savings effect of CO<sub>2</sub> interacted with precipitation patterns such that while early growing season soil water was generally greater in years following CO<sub>2</sub> enrichment, the degree of this carry-over effect varied among years, with early-season soil water content being most affected by CO<sub>2</sub> treatment in 1998, and unaffected in 1999 (Figure 1). Such carry-over effects of high soil water content are undoubtedly important for early-season

plant growth, when WUE tends to be highest due to cool temperatures, and may have contributed to the increased WUE in the high CO<sub>2</sub> treatment in the average rainfall year 1998 (Figure 6), as well as enhancing the  $\Psi_{\text{leaf}}$  responsiveness to CO<sub>2</sub> enrichment that same year (Table 1). Using this same argument, soil water contents were highest in all treatments at the start of the 1998 growing season (Figure 1), a condition which may account for WUE also being highest that year compared to all other study years ( $P < 0.05$ ; when averaged across all CO<sub>2</sub> treatments). These observations stress the need to consider multiple years in interpreting plant biomass production and water use responses of the SGS to CO<sub>2</sub> enrichment or any other treatments that interact with soil/plant water dynamics.

This study reports substantial and consistent improvement in soil and plant water relations in elevated vs. ambient CO<sub>2</sub> open top chambers. However, the interpretation of these responses for a future CO<sub>2</sub> enriched world is complicated by the use of this open top chamber CO<sub>2</sub> enrichment system, which caused changes in the plot microclimate. Higher ambient temperatures are likely one of the more important environmental perturbations in open top chamber experiments. Chamber warming was implicated in the 36% enhanced biomass production observed in chambered-ambient compared to non-chambered plots of the present study. Morgan et al., (in press) attributed this warming effect to a lengthened growing season and increased N mineralization. Open top chambers also have the potential to alter system hydrology through changes in the energy balance (Ham et al., 1995). In the present study, soil moisture was typically lower in ambient CO<sub>2</sub> plots compared to unchambered control plots (Figure 1), a response attributed to greater plant biomass, and therefore, greater transpirational surface inside compared to outside chambers (Morgan et al., in press). However,  $\Psi_{\text{leaf}}$  in these shortgrass species were similar between the unchambered controls and the drier, chambered ambient CO<sub>2</sub> grown plants, suggesting that the chamber environment may have been beneficial for plant water relations. Further, WUE was higher in chambered ambient CO<sub>2</sub> plots compared to unchambered controls (Figure 6). Ham et al., (1995) reported that open top chambers in the tallgrass prairie of Kansas altered the energy and water balance of plants such that plant and soil water relations were enhanced relative to unchambered field plots. Leaf water potentials of the dominant tallgrass, *Andropogon gerardii* (Owensby et al., 1993; Knapp et al., 1993), and several other tallgrass prairie species (Knapp et al.,

1996), and soil water content (Owensby et al., 1999), were consistently higher in chambered ambient CO<sub>2</sub> plots compared to unchambered field plots. The chamber effect on aboveground biomass was decidedly less in the Kansas tallgrass experiment (Owensby et al., 1999), which may have prevented soil water depletion that occurred in the more productive chambered plots of the Colorado study. While these chamber effects indicate that caution should be used in the direct application of the data to future CO<sub>2</sub> enriched environments, the strong effects of CO<sub>2</sub> on plant production (Morgan et al., in press) and plant/soil water relations (present study) seen in comparisons between ambient and elevated CO<sub>2</sub> chamber treatments indicate a potentially strong effect of rising CO<sub>2</sub> levels on the shortgrass steppe through altered water relations.

In the water-limited SGS, elevated CO<sub>2</sub> enhances plant water relations of both C<sub>3</sub> and C<sub>4</sub> grass species, improves WUE and leads to increased soil moisture during large portions of the growing season. This increase in soil moisture has been shown to be the major controlling factor in improved C assimilation rates and increased total aboveground biomass in this system (Morgan et al., 2001, in press; LeCain et al., 2003) and will likely decrease the susceptibility of the SGS to drought. Likewise, increased water drainage under elevated CO<sub>2</sub> in combination with differing rooting patterns (e.g., shallow vs. deep) may allow some species to benefit more from deeper water storage which could ultimately lead to species compositional changes. These results have important consequences for ecosystem processes such as nutrient cycling and carbon storage in not only the shortgrass steppe (Pendall et al., in press) but also semi-arid ecosystems worldwide.

### Acknowledgements

The authors thank Dean Ackerman, Scott Andre, Barry Weaver and Dennis Mueller for designing and building the open top chambers and for technical assistance, and Clenton Owensby, Lisa Auen, Fred Caldwell and Doug Whitt for guidance on chamber design. We also thank Larry Tissue, Adam Fleener and David Jensen for TDR monitoring, Mary Ashby and Jeff Thomas for on-site logistical support, Jean Reeder for soils information at the site, Mark West for statistical advice and Justin Derner and Elise Pendall for critically reviewing the manuscript. This research was supported by funding from the Terrestrial Ecology and

Climate Change Initiative (grant NSF-IBN-9524068 and grant USDA/NRICGP-98-134) and from grant NSF DEB-9708596.

### References

- Arp W J, van Mierlo J E M, Berendse F and Snijders W 1998 Interactions between elevated CO<sub>2</sub> concentration, nitrogen and water: effects on growth and water use of six perennial plant species. *Plant Cell Environ.* 21, 1–11.
- Bremer D J, Ham J M and Owensby C E 1996 Effect of elevated atmospheric carbon dioxide and open-top chambers on transpiration in a tallgrass prairie. *J. Environ. Qual.* 25, 691–701.
- Chiariello N R and Field C B 1996 Annual grassland responses to elevated CO<sub>2</sub> in multiyear community microcosms. In *Carbon Dioxide, Populations, and Communities*. Eds. Ch. Körner and F A Bazzaz, pp. 139–157. Academic Press, New York.
- Drake B G, González-Meler M A and Long S P 1997 More efficient plants: A consequence of rising atmospheric CO<sub>2</sub>? *Annual Rev. Plant Physiol. Mol. Biol.* 48, 609–639.
- Epstein H E, Laurenroth W K, Burke I C and Coffin D P 1997 Productivity patterns of C<sub>3</sub> and C<sub>4</sub> functional types in the Great Plains of the U.S. *Ecology* 78, 722–731.
- Ferretti D F, E Pendall J A Morgan, J A Nelson, D R LeCain and A R Mosier 2003 Partitioning evapotranspiration fluxes from a Colorado grassland using stable isotopes: Seasonal variations and implication for elevated atmospheric CO<sub>2</sub>. *Plant Soil*, 254, 291–303.
- Field C B, Jackson R B and Mooney H A 1995 Stomatal responses to increased CO<sub>2</sub>: Implications from the plant to the global scale. *Plant Cell Environ.* 18, 1214–1225.
- Field C B, Lund C P, Chiariello N R and Mortimer B E 1997 CO<sub>2</sub> effects on the water budget of grassland microcosm communities. *Global Change Biol.* 3, 197–206.
- Freden A L, Randerson J T, Holbrook N M and Field C B 1997 Elevated atmospheric CO<sub>2</sub> increases water availability in a water-limited grassland ecosystem. *J. Am. Water Resour. Assoc.* 33, 1033–1039.
- Grünzweig J M and Körner Ch 2001 Growth, water and nitrogen relations in grassland model ecosystems of the semi-arid Negev of Israel exposed to elevated CO<sub>2</sub>. *Oecologia* 128, 251–262.
- Ham J M, Owensby C E, Coyne P I and Bremer D J 1995 Fluxes of CO<sub>2</sub> and water vapor from a prairie ecosystem exposed to ambient and elevated atmospheric CO<sub>2</sub>. *Ag. Forest Meteorol.* 77, 73–93.
- Jackson R B, Sala O E, Field C B and Mooney H A 1994 CO<sub>2</sub> alters water use, carbon gain, and yield for the dominant species in a natural grassland. *Oecologia* 98, 257–262.
- Knapp A K, Hamerlynck E P, Ham J M and Owensby C E 1996 Responses in stomatal conductance to elevated CO<sub>2</sub> in 12 grassland species that differ in growth form. *Vegetatio* 125, 31–41.
- Knapp A K, Hamerlynck E P and Owensby C E 1993 Photosynthetic and water relations responses to elevated CO<sub>2</sub> in the C<sub>4</sub> grass *Andropogon gerardii*. *Intl. J. Plant Sci.* 154, 459–466.
- Lauenroth W K and Milchunas D G 1991 The shortgrass steppe. In *Natural grasslands, Ecosystems of the world*, Vol. 8. Ed. R T Coupland, pp. 183–226. Elsevier, New York.
- LeCain D R, Morgan J A, Mosier A R, Nelson J A. 2003. Soil and Plant Water Relations Determine Photosynthetic Responses of C<sub>3</sub> and C<sub>4</sub> Grasses in a Semi-arid Ecosystem Under Elevated CO<sub>2</sub>. *Annals Bot.* 92(1), 41–52.

- Lockwood J G 1999 Is potential evapotranspiration and its relationship with actual evapotranspiration sensitive to elevated atmospheric CO<sub>2</sub> levels? *Clim. Change* 41, 193–212.
- Mearns L O 2000 Climatic change and variability. *In* *Climate Change and Global Crop Productivity*. Eds. K R Reddy and H F Hodges. pp. 7–35. CABI Publishing Co., Wallingford, Oxon, UK.
- Milchunas D L and Lauenroth W K 2001 Belowground primary production by carbon isotope decay and long-term root biomass dynamics. *Ecosystems* 4, 139–150.
- Morgan J A, LeCain D R, Mosier A R and Milchunas D G 2001 Elevated CO<sub>2</sub> enhances water relations and productivity and affects gas exchange in C<sub>3</sub> and C<sub>4</sub> grasses of the Colorado shortgrass steppe. *Glob. Change Biol.* 7, 451–466.
- Morgan, J A, A R Mosier, D G Milchunas, D R LeCain, J A Nelson and W J Parton. CO<sub>2</sub> enhances productivity of the shortgrass steppe, alters species composition and reduces forage digestibility. *Ecol. Applic.* (in press).
- Morison J I L 1985 Sensitivity of stomata and water use efficiency to high CO<sub>2</sub>. *Plant Cell Environ.* 8, 467–474.
- Morison J I L 1993 Response of plants to CO<sub>2</sub> under water limited conditions. *Vegetatio* 104/105, 193–209.
- Morison J I L and Gifford R M 1983 Stomatal sensitivity to carbon dioxide and humidity: A comparison of two C<sub>3</sub> and two C<sub>4</sub> grass species. *Plant Physiol.* 71, 789–796.
- Nie D, He H, Mo G, Kirkham M B and Kanemasu E T 1992 Canopy photosynthesis and evapotranspiration of rangeland plants under doubled carbon dioxide in closed-top chambers. *Ag. Forest Meteorol.* 61, 205–217.
- Niklaus P A, Spinnler D and Körner Ch 1998 Soil moisture dynamics of calcareous grassland under elevated CO<sub>2</sub>. *Oecologia* 117, 201–208.
- Owensby C E, Coyne P I, Ham J H, Auen L M and Knapp A K 1993 Biomass production in a tallgrass prairie ecosystem exposed to ambient and elevated CO<sub>2</sub>. *Ecol. Applic.* 3, 644–653.
- Owensby C E, Ham J M, Knapp A K and Auen L M 1999 Biomass production and species composition change in a tallgrass prairie ecosystem after long-term exposure to elevated atmospheric CO<sub>2</sub>. *Global Change Biol.* 5, 497–506.
- Pendall E, Del Grosso S, King J Y et al. Elevated atmospheric CO<sub>2</sub> effects and soil water feedbacks on soil respiration components in a Colorado grassland. *Glob. Biogeochem. Cycles* (in press).
- Polley H W, Mayeux H S, Johnson H B and Tischler C R 1997 Viewpoint: Atmospheric CO<sub>2</sub>, soil water, and shrub/grass ratios on rangelands. *J. Range Manage.* 50, 278–284.
- Sala O E, Lauenroth W K and Parton W J 1992 Long-term soil water dynamics in the shortgrass steppe. *Ecology* 73, 1175–1181.
- Sindhøj E, Hansson A C, Andréon O, Kätterer T, Marissink M and Pettersson R 2000 Root dynamics in a semi-natural grassland in relation to atmospheric carbon dioxide enrichment, soil water and shoot biomass. *Plant Soil* 223, 253–263.
- Singh J S, Milchunas D G and Lauenroth W K 1998 Soil water dynamics and vegetation patterns in a semiarid grassland. *Plant Ecol.* 134, 77–89.
- Tyree M T and Alexander J D 1993 Plant water relations and the effects of elevated CO<sub>2</sub>: A review and suggestions for future research. *Vegetatio* 104/105, 47–62.
- Volk M, Niklaus P A and Körner Ch 2000 Soil moisture effects determine CO<sub>2</sub> responses of grassland species. *Oecologia* 125, 380–388.
- Ward S J E, Midgley G F, Jones M H and Curtis P S 1999 Responses of wild C<sub>4</sub> and C<sub>3</sub> grass (Poaceae) species to elevated atmospheric CO<sub>2</sub> concentration: A meta-analytic test of current theories and perceptions. *Glob. Change Biol.* 5, 723–741.
- Weaver J E and Albertson F W 1956 *Grasslands of the Great Plains*. Johns. Lincoln, Nebraska.
- Wullschlegel S D, Tschaplinski T J and Norby R J 2002 Plant water relations at elevated CO<sub>2</sub> – implications for water-limited environments. *Plant Cell Environ.* 25, 319–331.

*Section editor: C. Neill*