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

7

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

3

4 **Abstract**

5 Increasing atmospheric [CO<sub>2</sub>] has potentially significant impacts on the dynamics of  
6 water use and conservation in semi-arid rangelands. In this study we used large (15.5 m<sup>2</sup>)  
7 open top chambers to investigate effects of twice ambient [CO<sub>2</sub>] on plant and soil water  
8 relations of semi-arid shortgrass steppe (SGS) of northeastern Colorado from 1997 to  
9 2001. Seasonal average soil moisture throughout the soil profile (0-15, 15-45, 45-75, 75-  
10 105 cm) was increased under elevated CO<sub>2</sub> compared to ambient CO<sub>2</sub> for much of the  
11 study period. When averaged across years, the greatest relative increase (elevated vs.  
12 ambient) in soil moisture occurred in the 75-105 cm depth increment (16.4%). Averaged  
13 over the study period, leaf xylem pressure potential ( $\Psi_{\text{leaf}}$ ) was enhanced 24 – 30% under  
14 elevated CO<sub>2</sub> in the major warm- and cool-season grass species of the SGS (*Bouteloua*  
15 *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  
16 degree of responsiveness in  $\Psi_{\text{leaf}}$  to elevated CO<sub>2</sub> did not differ between C<sub>3</sub> and C<sub>4</sub> plant  
17 functional types, but did differ between C<sub>3</sub> species. Water-use efficiency (WUE; g  
18 phytomass produced/ kg water consumed) was 43% higher on average in elevated (6.10)  
19 than ambient (4.27) CO<sub>2</sub> plots over the study period. Results suggest that a future,  
20 elevated CO<sub>2</sub> environment may result not only in increased plant productivity due to  
21 improved WUE, but also lead to increased water drainage and deep soil moisture storage  
22 in this semi-arid grassland ecosystem. This, along with the ability of the major grass  
23 species to maintain a favorable water status under elevated CO<sub>2</sub>, should result in the SGS

- 1 being less susceptible to prolonged periods of drought. However, species compositional
- 2 changes may occur with deeper-rooted species being favored over shallow-rooted
- 3 species.

## 1 **Introduction**

2 Grasslands are highly sensitive to changes in water supply, with small changes in water  
3 balance often causing large changes in ecosystem structure and function (Sala et al.  
4 1992). With atmospheric CO<sub>2</sub> concentrations projected to double by the end of this  
5 century (Mearns 2000), determining how elevated CO<sub>2</sub> may affect soil moisture in semi-  
6 arid environments will be crucial in predicting resulting grassland ecosystem structure,  
7 function and water use. Previous studies have reported increased soil moisture under  
8 elevated CO<sub>2</sub> in semi-arid, C<sub>3</sub>, annual grasslands in California (Fredeen et al. 1997),  
9 mesic, C<sub>3</sub>/C<sub>4</sub>, perennial tallgrass prairie in Kansas (Owensby et al. 1993, 1999; Ham et al.  
10 1995; Bremer et al. 1996), and mesic, C<sub>3</sub>, perennial grasslands in Switzerland (Niklaus et  
11 al. 1998) and Sweden (Sindhøj et al. 2000). How elevated CO<sub>2</sub> will affect the water  
12 balance in semi-arid rangeland with mixed C<sub>3</sub>/C<sub>4</sub> perennial vegetation such as that found  
13 in the shortgrass steppe of North America is unknown.

14       Effects of elevated CO<sub>2</sub> on water conservation at the leaf level are fairly well  
15 understood, with most studies demonstrating that, in drier conditions under elevated CO<sub>2</sub>,  
16 leaf water-use efficiency is improved (Morison 1993, Owensby et al. 1993, Jackson et al.  
17 1994). However, effects of elevated CO<sub>2</sub> on ecosystem water balance needs more  
18 attention (Owensby et al. 1999). For conservation of water at the ecosystem-level to  
19 occur, the reduction in water loss per unit leaf area under elevated CO<sub>2</sub> must not be offset  
20 by an increase in total evaporative leaf surface (Field et al. 1997, Niklaus et al. 1998,  
21 Volk et al. 2000) and reduced stand evapotranspiration (ET) must be evident (Drake et al.  
22 1997). Direct observations of increased soil moisture under elevated CO<sub>2</sub> are primarily  
23 limited to grassland ecosystems (Field et al. 1995) where availability of water is often

1 limiting and effects of stomatal closure on transpiration are not negated by an increase in  
2 leaf area (Polley et al. 1997, Lockwood 1999). Consequently, reduced ET under elevated  
3 CO<sub>2</sub> has also mostly been observed in grassland ecosystems (Nie et al. 1992, Ham et al.  
4 1995, Bremer et al. 1996, Fredeen et al. 1997, Field et al. 1997).

5         Decreased stomatal conductance ( $g_s$ ) under elevated CO<sub>2</sub> is often associated with  
6 increased leaf xylem pressure potential ( $\Psi_{leaf}$ ) which reflects improved plant water status  
7 and increased drought tolerance (Tyree and Alexander 1993). Mechanisms that  
8 contribute to improved  $\Psi_{leaf}$  under elevated CO<sub>2</sub> are not fully known but have been  
9 attributed to reduced  $g_s$  and increased plant hydraulic conductance along with improved  
10 leaf turgor associated with osmotic adjustment (Tyree and Alexander 1993, Wullschlegel  
11 et al. 2002). There appears to be little difference in stomatal sensitivity to elevated CO<sub>2</sub>  
12 between C<sub>3</sub> and C<sub>4</sub> species (Morison and Gifford 1983, Polley et al. 1997, Wand et al.  
13 1999). However, direct comparisons of the degree to which  $\Psi_{leaf}$  is altered by elevated  
14 CO<sub>2</sub> among naturally co-occurring C<sub>3</sub> and C<sub>4</sub> grass species have not been conducted.

15         Water is the most important factor that governs ecosystem structure and function  
16 in the semi-arid shortgrass steppe (SGS) of North America (Sala et al. 1992). Large  
17 temporal variability in rainfall and the preponderance of small precipitation events in this  
18 region increase the importance of soil water stored below the evaporative zone for the  
19 perpetuation and stability of perennial shortgrass vegetation (Singh et al. 1998). Any  
20 substantial increase in soil moisture under elevated CO<sub>2</sub> or an increase in the percolation  
21 of precipitation deeper into the soil profile (Grünzweig and Körner 2001) could have a  
22 considerable impact on the production and survival of SGS species during prolonged  
23 periods of drought.

1           In this study, we used large (15.5 m<sup>2</sup>) open top chambers to examine the effects of  
2 elevated CO<sub>2</sub> on plant water relations of coexisting C<sub>3</sub> and C<sub>4</sub> grass species, ecosystem  
3 water use efficiency and soil moisture dynamics in the SGS of northeastern Colorado.  
4 Specifically, we tested the hypotheses that 1) elevated CO<sub>2</sub> will increase soil water  
5 content, especially in lower soil depths that are free from evaporative demand, 2)  
6 elevated CO<sub>2</sub> will increase  $\Psi_{\text{leaf}}$  in three important grass species of the SGS, 3) the degree  
7 of responsiveness in  $\Psi_{\text{leaf}}$  to elevated CO<sub>2</sub> will be the same between C<sub>3</sub> and C<sub>4</sub> grass  
8 species and 4) water-use efficiency (g of aboveground phytomass produced / kg of water  
9 consumed during the growing season) will be greater under elevated CO<sub>2</sub>.

10

## 11 **Methods**

### 12 *Study site*

13 Experiments were conducted at the USDA-ARS Central Plains Experimental Range  
14 (CPER) located in the northern portion of the shortgrass steppe (SGS) about 60 km  
15 northeast of Fort Collins, Colorado (40°49'N, 107°47'W; elevation 1650 m). Mean  
16 annual precipitation averaged 320 mm over the past 55 years with about 80% occurring  
17 between April and September (Lauenroth and Milchunas 1991). Long-term monthly  
18 average air temperatures range from minus 5 °C in January to 22 °C in July. The study  
19 site is located about 20 km south of the continental transition between C<sub>3</sub> and C<sub>4</sub>  
20 dominance (Epstein et al. 1997). Vegetation is dominated by *Bouteloua gracilis* (H.B.K.)  
21 Lag. (blue grama) a C<sub>4</sub> grass, but C<sub>3</sub> grasses *Pascopyrum smithii* (Rydb.) A. Love  
22 (western wheatgrass) and *Stipa comata* Trin and Rupr. (needle and thread) are also a

1 major vegetation component. Soil at the experimental site is a Remmit fine sandy loam  
2 (Ustollic camborthids).

3

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

5 Large (15.5 m<sup>2</sup>) open top chambers were used to investigate effects of elevated CO<sub>2</sub> on  
6 native vegetation of the SGS. The experiment was established during the spring of 1997  
7 on a six ha field of native rangeland with a mixture of cool- and warm-season grass  
8 species. The field was divided into three blocks based on uniformity of vegetation; three  
9 15.5 m<sup>2</sup> circular plots were randomly chosen as experimental plots within each block.  
10 Open top chambers were placed on two plots in each of the three blocks (six total) from  
11 late March until mid-October of each year. Within each block, one chamber was  
12 randomly assigned an ambient CO<sub>2</sub> treatment (~360 μmol mol<sup>-1</sup>), the other an elevated  
13 CO<sub>2</sub> treatment (~720 μmol mol<sup>-1</sup>). Each block also had an unchambered plot of equal  
14 ground area, which was used to monitor effects of the chambers. See Morgan et al.  
15 (2001) for a detailed description of experimental and open top chamber design.

16 Elevated CO<sub>2</sub> chambers were maintained at a concentration of 720 ± 15 μmol  
17 mol<sup>-1</sup> by injecting 100% CO<sub>2</sub> into input fans where it was mixed with ambient air before  
18 being delivered into chambers. CO<sub>2</sub> concentration inside chambers was monitored  
19 regularly by drawing air from a sampling manifold, positioned 2 m high across the center  
20 of chambers, to an infra-red gas analyzer (LI-COR LI6262; LI-COR, Lincoln, NE, USA).  
21 Soil within chambers was isolated by an aluminum flange buried 0.8 m deep around the  
22 outside edge of chamber walls. Openings in the top of each chamber were reduced to  
23 0.75 m and incident precipitation upon the chamber tops was channeled into reservoirs



1 and immediately pumped onto chambered plots using an automated sprinkler system.  
2 The precipitation catching system was not 100% efficient, so the amount of applied water  
3 was carefully monitored with flow meters and deficiencies were made up at least weekly.  
4 Consequently, chambered plots (ambient and elevated CO<sub>2</sub>) and unchambered controls  
5 received the same total amount of precipitation.

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

15

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

17 Volumetric soil moisture content was measured from an access tube within each plot on a  
18 weekly basis using a Troxler model 4301 neutron probe (Troxler Electronics Lab.,  
19 Research Triangle Park, NC, USA) which had been calibrated against soil at the  
20 experimental site. Time Domain Reflectometry (TDR) was also used weekly to measure  
21 soil moisture in the top 15 cm of the soil profile where neutron scattering (under varying  
22 moisture conditions) may reduce accuracy of neutron probe measurements. Depth

1 increments for soil moisture analyses were 0-15 (TDR), 15-45, 45-75 and 75-105 cm  
2 (neutron probe placed in center of each depth increment).

3 Leaf xylem pressure potential ( $\Psi_{\text{leaf}}$ ) was measured weekly throughout each  
4 growing season (April-October) on 1-2 leaves each of *B. gracilis*, *P. smithii* and *S.*  
5 *comata* in all plots with a Scholander-type pressure chamber (PMS Instrument Company,  
6 Corvallis, OR, USA). Measurements were taken mid-morning (1000-1145 hrs MST)  
7 when plants were typically most active and sampled leaves were processed immediately  
8 after being cut. Leaves of *P. smithii* and *S. comata* were on average large enough to  
9 sample 1-2 weeks before those of *B. gracilis* in late-April each year except in 2000 when  
10 a severe drought delayed growth of the C<sub>3</sub> species.

11

### 12 *Water-use efficiency*

13 Aboveground plant biomass harvests were conducted in the southern half of all chambers  
14 at two different times during each growing season. Vegetation was clipped to crown-  
15 level from half of the harvest area (3.46 m<sup>2</sup> total area) in late July, which coincides with  
16 peak standing crop. Vegetation from the remaining half of the harvest area was clipped  
17 in late October for an estimate of total seasonal productivity. The area harvested at peak  
18 standing crop was re-clipped in October to estimate regrowth. Total seasonal  
19 aboveground production was the sum of all harvested biomass. Water-use efficiency was  
20 calculated as the total aboveground biomass (g) produced per total amount of water (kg)  
21 lost from the same area during the same time period. Total water lost was calculated by  
22 subtracting season-ending soil water and precipitation from season-beginning soil water.  
23 We assumed there was minimal run-off and that percolation of water rarely reached

1 below 105 cm (deepest neutron probe reading) because of coarse-textured soils and  
2 preponderance of small rainfall events.

3

#### 4 *Statistical analyses*

5 The fixed effects of CO<sub>2</sub> treatment on seasonal soil water content,  $\Psi_{\text{leaf}}$ , species  $\Psi_{\text{leaf}}$   
6 responsiveness and WUE were analyzed using the SAS PROC MIXED analysis (SAS  
7 Institute Inc., Cary, NC, USA), with year represented as a repeated measure and block  
8 designated as a random effect. Means comparisons were made using Least Squares  
9 Means (Tukey's HSD p-value adjustment) with differences reported as significant when  
10  $P < 0.05$ .

11

## 12 **Results**

### 13 *Seasonal climate and chamber environment*

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

21 Air temperature was on average 2.6 °C higher inside the chambers than outside,  
22 and soil temperature averaged 1.25 °C higher in the chambers. Lexan walls of the  
23 chambers caused a 6-8% reduction in PAR. However, daily PAR was reduced about

1 28% inside the chambers compared to the outside environment, with the majority of this  
2 reduction attributed to shading from the chamber framework.

3

#### 4 *Soil water content*

5 Volumetric soil water content in the 15-45 and 75-105 cm depth increments was higher  
6 under elevated CO<sub>2</sub> compared to ambient CO<sub>2</sub> over the course of the study period, with a  
7 strong trend (P = 0.08) for higher soil water content at 45-75 cm (Fig. 2). The significant  
8 treatment \* year interaction at the 0-15 cm depth indicated that soil water content under  
9 elevated CO<sub>2</sub> was higher than ambient CO<sub>2</sub> in 1999 only. Soil water content in control  
10 plots was similar to elevated CO<sub>2</sub> plots at all depths for the entire study (Fig. 2). The  
11 significant year effect at the three lower depths indicated that soil water content was  
12 highest in 1999 and lowest in 2001. When averaged over all depths and years,  
13 volumetric soil water content was 12.1% in elevated CO<sub>2</sub> and 10.7% in ambient CO<sub>2</sub>  
14 plots. This difference was significant even though the data included periods when there  
15 were no differences in soil water content (Fig. 1).

16

#### 17 *Midday leaf water potential*

18 Seasonal average midday leaf water potential ( $\Psi_{\text{leaf}}$ ) of three common perennial grass  
19 species of the SGS was higher under elevated CO<sub>2</sub> compared to ambient CO<sub>2</sub> and control  
20 grown plants for the majority of this study (Fig. 3). Significant treatment \* year  
21 interactions in all three species indicated that *Stipa comata* (C<sub>3</sub>) had higher  $\Psi_{\text{leaf}}$  under  
22 elevated CO<sub>2</sub> for all five years of the study while *Pascopyrum smithii* (C<sub>3</sub>) and *Bouteloua*

1 *gracilis* (C<sub>4</sub>) had higher seasonal  $\Psi_{\text{leaf}}$  in all but the first year of the study. Seasonal  
2 midday  $\Psi_{\text{leaf}}$  did not differ between ambient CO<sub>2</sub> and unchambered control plots.

3 The degree of responsiveness of  $\Psi_{\text{leaf}}$  to elevated CO<sub>2</sub>, or the percent increase in  
4  $\Psi_{\text{leaf}}$  in elevated CO<sub>2</sub> versus ambient CO<sub>2</sub> plants, indicated significant species ( $P = 0.02$ )  
5 and year ( $P < 0.0001$ ) effects (Table 1). *Stipa comata* was more responsive to elevated  
6 CO<sub>2</sub> than its C<sub>3</sub> counterpart *P. smithii*. However, neither of the C<sub>3</sub> species differed from  
7 the C<sub>4</sub> grass *B. gracilis* in their  $\Psi_{\text{leaf}}$  responsiveness to elevated CO<sub>2</sub>. Averaged across  
8 species, the responsiveness of  $\Psi_{\text{leaf}}$  to elevated CO<sub>2</sub> was higher in 1998 with the  
9 remaining four years similar.

10

#### 11 *Water-use efficiency*

12 Water-use efficiency was increased under elevated CO<sub>2</sub> compared to ambient CO<sub>2</sub> in  
13 1998 and 2000, with strong trends for increased WUE in the remaining three years (Fig.  
14 4). The greatest increase in WUE (74%) with elevated CO<sub>2</sub> was observed in the drought  
15 year of 2000. For this study, WUE averaged 6.10 g kg<sup>-1</sup> under elevated CO<sub>2</sub> and was  
16 higher than the 4.27 g kg<sup>-1</sup> observed in ambient CO<sub>2</sub> plots. Control plots exhibited the  
17 lowest WUE across years at 2.82 g kg<sup>-1</sup>. Total amount of water consumed over the course  
18 of each growing season differed very little between treatments (data not shown); thus  
19 differences in WUE between treatments resulted from large differences in biomass  
20 between treatments (Morgan et al. 2001).

21

## 1 **Discussion**

2           Occupying the western edge of the central Great Plains of North America, the  
3 shortgrass steppe (SGS) is an ecosystem governed by water, and the impact that elevated  
4 CO<sub>2</sub> has on water availability will largely determine the response of this system to future  
5 increased atmospheric [CO<sub>2</sub>]. Potential evaporation always exceeds precipitation in this  
6 semi-arid environment (Lauenroth and Milchunas 1991, Sala et al. 1992) and water  
7 stored below the evaporative zone is extremely important in stabilizing this plant  
8 community during prolonged periods of drought (Singh et al. 1998). Over the course of  
9 this study we found that soil moisture under elevated CO<sub>2</sub> was increased by 11-16% in  
10 soil depth increments at (15-45 cm) or below (45-75, 75-105 cm) the evaporative zone.  
11 These increases are striking considering they occurred over a relatively long time frame  
12 which included drought periods when soil water content between elevated CO<sub>2</sub> and  
13 ambient CO<sub>2</sub> plots did not differ (Fig. 1). Also, soil moisture below the evaporative zone  
14 under elevated CO<sub>2</sub> was similar to that in the unchambered controls (Fig. 2) even though  
15 the elevated CO<sub>2</sub> plots were on average 2.6 °C warmer and supported 120% more  
16 aboveground biomass (Morgan et al. unpubl data).

17           Elevated CO<sub>2</sub> increased soil moisture in the uppermost soil (0-15 cm) depth  
18 increment only in a very wet year (1999). High evaporative demand in this surface soil  
19 layer may have offset any direct effect of reduced plant transpiration on soil moisture  
20 under CO<sub>2</sub> enrichment, even though stable isotope partitioning of ET in the SGS has  
21 shown that the evaporation component of ET is significantly lower under elevated CO<sub>2</sub>  
22 (Ferretti et al. unpubl data). Increased soil moisture under elevated CO<sub>2</sub> at the deepest  
23 soil depth indicates that water percolated deeper into the soil profile and that the rate of

1 precipitation lost to plant transpiration and/or soil evaporation was less under elevated  
2 CO<sub>2</sub>. These results support our hypothesis that the relative increase in soil moisture  
3 under elevated CO<sub>2</sub> would be greatest at the lower soil depths where water can be stored  
4 free from evaporative demand and in a less densely rooted area. While some reports have  
5 suggested that increased soil drainage under elevated CO<sub>2</sub> has a negative effect on water  
6 balance in shallow soils (Grünzweig and Körner 2001), this phenomenon enhances water  
7 storage in the deep fine sandy loam soils underlying large portions of the SGS. On  
8 average, 67% of production in the SGS is belowground (Milchunas and Lauenroth 2001),  
9 and competition for water is a major key to survival. Rooting patterns of three major  
10 grass species (*B. gracilis*, *P. smithii*, *S. comata*; comprise approximately 88% of total  
11 aboveground biomass) are quite different (Weaver and Albertson 1956), reducing  
12 competition for water and increasing coexistence among these species. However, species  
13 with deeper root systems, *P. smithii* (deeply rooted) and *S. comata* (shallow to  
14 intermediately rooted), may benefit more from deep soil moisture storage under elevated  
15 CO<sub>2</sub> than the shallow rooted *B. gracilis* eliciting species compositional changes.

16 We hypothesized that  $\Psi_{\text{leaf}}$  would be enhanced in all three of our study species due  
17 to CO<sub>2</sub>-induced increases in soil water content. Indeed, seasonal  $\Psi_{\text{leaf}}$  was higher in *B.*  
18 *gracilis* and *P. smithii* in 4 out of the 5 years, and higher in all 5 years in *S. comata*.  
19 Further,  $\Psi_{\text{leaf}}$  of *S. comata* was more responsive to changes in CO<sub>2</sub> than the deeper rooted  
20 *P. smithii*. It seems plausible that  $\Psi_{\text{leaf}}$  of the deeper rooted *P. smithii*, with a more steady  
21 soil water supply, would be less sensitive to CO<sub>2</sub>-induced changes in soil water content  
22 than *S. comata*. Although the most consistent CO<sub>2</sub>-induced changes in soil water content  
23 occurred at the deepest depths, it is likely that the ecological effects of soil water content

1 differences may have been at least as important at intermediate soil depths where soil  
2 water was more limiting, and roots of *S. comata* more abundant. Regardless of the  
3 mechanism, it does indicate that *S. comata* has the ability to increase plant water status to  
4 a greater degree under elevated CO<sub>2</sub> over a wide range of soil moisture contents. This  
5 may possibly lead to increased leaf turgor and allow *S. comata* to continue growth further  
6 into periods of drought. Indeed, of these three grass species, *S. comata* was the only one  
7 to have a significant aboveground productivity response to elevated CO<sub>2</sub> over the  
8 course of this study (Morgan et al. unpubl data).

9         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>  
10 grasses. Although there are reasons to suggest that differences in stomatal behavior  
11 between C<sub>3</sub> and C<sub>4</sub> grasses (Morison and Gifford 1983, Morison 1985, Polley et al. 1997,  
12 Wand et al. 1999) might elicit different relationships between CO<sub>2</sub>-induced changes in  
13 leaf conductance (Drake et al. 1997), and therefore  $\Psi_{\text{leaf}}$ , that mechanism is likely  
14 unimportant in the SGS where differences in rooting depth among dominant and sub-  
15 dominant species may govern adaptability and individual plant water relations.

16         In dry grasslands, enhanced aboveground production under elevated CO<sub>2</sub> has been  
17 attributed primarily to improved water relations (Owensby et al. 1993, Jackson et al.  
18 1994, Chiariello and Field 1996, Morgan et al. 2001). Here, soil moisture increased  
19 under elevated CO<sub>2</sub> over the course of the study (Fig. 2), treatment differences occurred  
20 in soil water content at the end of three out of five growing seasons (Fig. 1), and  
21 consistently higher  $\Psi_{\text{leaf}}$  was observed under elevated CO<sub>2</sub> throughout the study (Fig. 3).  
22 Averaged over all five years, weekly  $\Psi_{\text{leaf}}$  of the dominant C<sub>4</sub> grass (*B. gracilis*) and two  
23 dominant C<sub>3</sub> grasses (*P. smithii* and *S. comata*) increased 24-30% under elevated CO<sub>2</sub>.



1 These results are similar to studies involving other C<sub>3</sub> and C<sub>4</sub> grass species (Owensby et  
2 al. 1993, Jackson et al. 1994). Leaf water potential in all three species was enhanced  
3 throughout each growing season, regardless of differences in soil moisture between  
4 ambient and elevated CO<sub>2</sub> plots. Mechanisms contributing to increased  $\Psi_{\text{leaf}}$  under  
5 elevated CO<sub>2</sub> may include decreased  $g_s$ , increased plant hydraulic conductance and  
6 osmotic adjustment (Tyree and Alexander 1993, Wullschleger et al. 2002). Stomatal  
7 conductance of *B. gracilis* and *P. smithii* leaves was reduced by 27-36% and leaf  
8 transpiration efficiency was increased by 75-80% over four years of elevated CO<sub>2</sub> in the  
9 SGS (LeCain et al. unpubl data).

10 Elevated CO<sub>2</sub> increased WUE from 31-74% compared to ambient CO<sub>2</sub>. This  
11 result concurs with those in a recent review (Wullschleger et al. 2002) which found that  
12 most studies reported a 30-50% increase in WUE with elevated CO<sub>2</sub>. Whole-plant WUE  
13 ranged from no effect to a 180% increase under elevated CO<sub>2</sub> over a wide range of  
14 ecosystems and individual plant species (Wullschleger et al. 2002). In general, WUE  
15 appears affected more by elevated CO<sub>2</sub> under drought conditions (i.e., Field et al. 1997,  
16 Arp et al. 1998), which is supported by our results in 2000. However, the smallest  
17 increase in WUE due to CO<sub>2</sub> enrichment was measured in 2001 which, while having  
18 slightly above average precipitation, had the lowest recorded soil moisture during this  
19 study. This implies that the highly variable timing of precipitation in the SGS (Lauenroth  
20 and Milchunas 1991, Sala et al. 1992) may be an important factor in plant productivity  
21 and water relations responses of this system to elevated CO<sub>2</sub>. Indeed, the increase in both  
22 WUE averaged across CO<sub>2</sub> treatment and  $\Psi_{\text{leaf}}$  responsiveness to CO<sub>2</sub> enrichment was

1 greatest in an average rainfall year (1998), but the timing of precipitation in 1998 may  
2 have been more favorable for plant water relations and production responses.

3         This study reports substantial and consistent improvement in soil and plant water  
4 relations in elevated vs. ambient CO<sub>2</sub> open top chambers. However, the interpretation of  
5 these responses for a future CO<sub>2</sub> enriched world may be complicated by the use of this  
6 CO<sub>2</sub> enrichment system. Ham et al. (1995) reported that open top chambers in the  
7 tallgrass prairie of Kansas altered the energy and water balance of plants such that plant  
8 and soil water relations were enhanced relative to unchambered field plots. Leaf water  
9 potentials of the dominant tallgrass, *Andropogon gerardii* (Owensby et al. 1993, Knapp  
10 et al. 1993), and several other tallgrass prairie species (Knapp et al. 1996), and soil water  
11 content (Owensby et al. 1999), were consistently higher in chambered ambient CO<sub>2</sub> plots  
12 compared to unchambered field plots. In this study, soil moisture was typically lower in  
13 ambient CO<sub>2</sub> plots compared to unchambered control plots (Fig. 1). However,  $\Psi_{\text{leaf}}$  in  
14 these shortgrass species were similar between chambered ambient CO<sub>2</sub> grown plants and  
15 unchambered controls, suggesting that the chamber environment may have enhanced  $\Psi_{\text{leaf}}$   
16 in the drier chambered plots. Likewise, WUE was higher in chambered ambient CO<sub>2</sub>  
17 plots compared to unchambered controls (Fig. 4).

18         In the water-limited SGS, elevated CO<sub>2</sub> enhances plant water relations of both C<sub>3</sub>  
19 and C<sub>4</sub> grass species, improves WUE and leads to increased soil moisture during large  
20 portions of the growing season. This increase in soil moisture has been shown to be the  
21 major controlling factor in improved C assimilation rates and increased total  
22 aboveground biomass in this system (Morgan et al. 2001) and will likely decrease the  
23 susceptibility of the SGS to drought. Likewise, increased water drainage under elevated

1 CO<sub>2</sub> in combination with differing rooting patterns (e.g., shallow vs. deep) may allow  
2 some species to benefit more from deeper water storage which could ultimately lead to  
3 species compositional changes. These results have important consequences for  
4 ecosystem processes such as nutrient cycling and carbon storage in not only the  
5 shortgrass steppe (Pendall et al. in press) but also semi-arid ecosystems worldwide.

6

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1 **Figure captions**

2

3 Figure 1. Total soil water content (mm) in the upper 1m of the soil profile in  
4 unchambered controls and chambered ambient (360  $\mu\text{mol mol}^{-1}$ ) and elevated (720  $\mu\text{mol}$   
5  $\text{mol}^{-1}$ ) CO<sub>2</sub> plots in five growing seasons (1997-2001) on the shortgrass steppe of  
6 Colorado. Total growing season precipitation (mm) is noted for each year of the study.

7

8 Figure 2. Seasonal average (1997-2001) percent volumetric soil water content ( $\pm 1$  S.E.;  
9  $n=3$ ) of the 0-15 cm, 15-45 cm, 45-75 cm and 75-105 cm depth increments in  
10 unchambered control and chambered ambient (360  $\mu\text{mol mol}^{-1}$ ) and elevated (720  $\mu\text{mol}$   
11  $\text{mol}^{-1}$ ) CO<sub>2</sub> plots on the shortgrass steppe of Colorado. Panel insets of analysis of  
12 variance results show main effects for each depth. Different letters on the 0-15 cm panel  
13 denote significant treatment effects ( $P<0.05$ ; Least Square Means) within each year as  
14 called for by the significant treatment \* year interaction. Significant treatment and year  
15 effects at the lower three depths are discussed in the text.

16

17 Figure 3. Seasonal average (1997-2001) midday (1000-1200 hrs MST) leaf water  
18 potential ( $\pm 1$  S.E.;  $n=3$ ) for three common grass species of the shortgrass steppe of  
19 Colorado in unchambered control and chambered ambient (360  $\mu\text{mol mol}^{-1}$ ) and elevated  
20 (720  $\mu\text{mol mol}^{-1}$ ) CO<sub>2</sub> plots. Different letters denote significant treatment effects  
21 ( $P<0.05$ ; Least Square Means) within a year for each grass species.

22

1 Figure 4. Yearly (1997-2001) water-use efficiency (g phytomass produced kg<sup>-1</sup> water  
2 consumed;  $\pm$  1 S.E.; n=3) for unchambered control and chambered ambient (360  $\mu$ mol  
3 mol<sup>-1</sup>) and elevated (720  $\mu$ mol mol<sup>-1</sup>) CO<sub>2</sub> plots in the shortgrass steppe of Colorado.  
4 Different letters denote significant treatment effects (P<0.05; Least Square Means) within  
5 a year.

6

7