Annals of Botany **92**: 41–52, 2003
doi:10.1093/aob/mcg109, available online at www.aob.oupjournals.org

Soil and Plant Water Relations Determine Photosynthetic Responses of C_3 and C_4 Grasses in a Semi-arid Ecosystem under Elevated CO_2

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Received: 2 December 2002 Returned for revision: 28 January 2003 Accepted: 12 March 2003 Published electronically: 9 May 2003

To model the effect of increasing atmospheric CO_2 on semi-arid grasslands, the gas exchange responses of leaves to seasonal changes in soil water, and how they are modified by CO_2, must be understood for C_3 and C_4 species that grow in the same area. In this study, open-top chambers were used to investigate the photosynthetic and stomatal responses of *Pascopyrum smithii* (C_3) and *Bouteloua gracilis* (C_4) grown at 360 (ambient CO_2) and 720 μmol mol^{-1} CO_2 (elevated CO_2) in a semi-arid shortgrass steppe. Assimilation rate (A) and stomatal conductance (g_s) at the treatment CO_2 concentrations and at a range of intercellular CO_2 concentrations and leaf water potentials (ψ_{leaf}) were measured over 4 years with variable soil water content caused by season and CO_2 treatment. Carboxylation efficiency of ribulose bisphosphate carboxylase/oxygenase ($V_{c,max}$), and ribulose bisphosphate regeneration capacity (J_{max}) were reduced in *P. smithii* grown in elevated CO_2, to the degree that A was similar in elevated and ambient CO_2 (when soil moisture was adequate). Photosynthetic capacity was not reduced in *B. gracilis* under elevated CO_2, but A was nearly saturated at ambient CO_2. There were no stomatal adaptations independent of photosynthetic acclimation. Although photosynthetic capacity was reduced in *P. smithii* growing in elevated CO_2, reduced g_s and transpiration improved soil water content and ψ_{leaf} in the elevated CO_2 chambers, thereby improving A of both species during dry periods. These results suggest that photosynthetic responses of C_3 and C_4 grasses in this semi-arid ecosystem will be driven primarily by the effect of elevated CO_2 on plant and soil water relations. © 2003 Annals of Botany Company

Key words: *Bouteloua gracilis*, *Pascopyrum smithii*, C_3, C_4, leaf water potential, photosynthesis, acclimation, stomata, semi-arid, soil water.

INTRODUCTION

Arid and semi-arid ecosystems occupy 40 % of the Earth's land surface (Dregne, 1991), so an understanding of the responses of these biomes to increasing atmospheric CO_2 is critical. Water-limited ecosystems are predicted to have relatively large responses to increasing CO_2 because elevated CO_2 decreases stomatal conductance and leaf transpiration, thereby improving soil water content (Volk *et al.*, 2000). A complicating factor in predicting the responses of grasslands to increasing CO_2 is that many grasslands have co-occuring C_3 and C_4 photosynthesis species (Wand *et al.*, 1999). Ehleringer *et al.* (1997) proposed that the global distribution of C_3 and C_4 plants was related to inherent differences in the quantum yield of photosynthesis. In theory, quantum yield is improved in C_3 plants as CO_2 increases, potentially expanding the global distribution of C_3 species. But the influence of elevated CO_2 on photosynthesis may be less important in arid ecosystems where the effects of water may dominate. An understanding of how seasonal and CO_2-induced variation in soil water affects photosynthetic and stomatal responses of competing C_3 and C_4 species is needed to predict the responses of arid grasslands to increasing CO_2.

Although carbon assimilation is stimulated relatively more in C_3 than in C_4 species when exposed to short-term increases in CO_2, this increase is often not maintained in plants exposed to elevated CO_2 for weeks or months (Sage, 1994; Moore *et al.*, 1999; Stitt and Krapp, 1999). This phenomenon has been termed 'photosynthetic acclimation'. The physiological basis for photosynthetic acclimation is not completely understood, but is often related to reduced leaf nitrogen and photosynthetic enzyme activity (Moore *et al.*, 1999; Stitt and Krapp, 1999). Little research has been done on possible stomatal acclimation to elevated CO_2, though reduced stomatal conductance and leaf transpiration may significantly affect ecosystem processes (Drake *et al.*, 1997; Jarvis *et al.*, 1999; Lee *et al.*, 2001).

Field studies are necessary to ascertain the responses of plants to elevated CO_2. There are few field studies in which the effects of elevated CO_2 on grasslands have been investigated, and even fewer studies in which C_3 and C_4 species occur in the same ecosystem (Wand *et al.*, 1999). There was no evidence of photosynthetic acclimation in C_3 or C_4 grasses in a tallgrass prairie (Nie *et al.*, 1992; Knapp *et al.*, 1993), a Texas grassland (Anderson *et al.*, 2001) or in a C_3 annual grassland (Jackson *et al.*, 1995). Conversely, photosynthetic acclimation was reported in both C_3 and C_4 species in a Minnesota grassland, and in C_3 grasses, but not a C_4 grass, in New Zealand (Lee *et al.*, 2001; von

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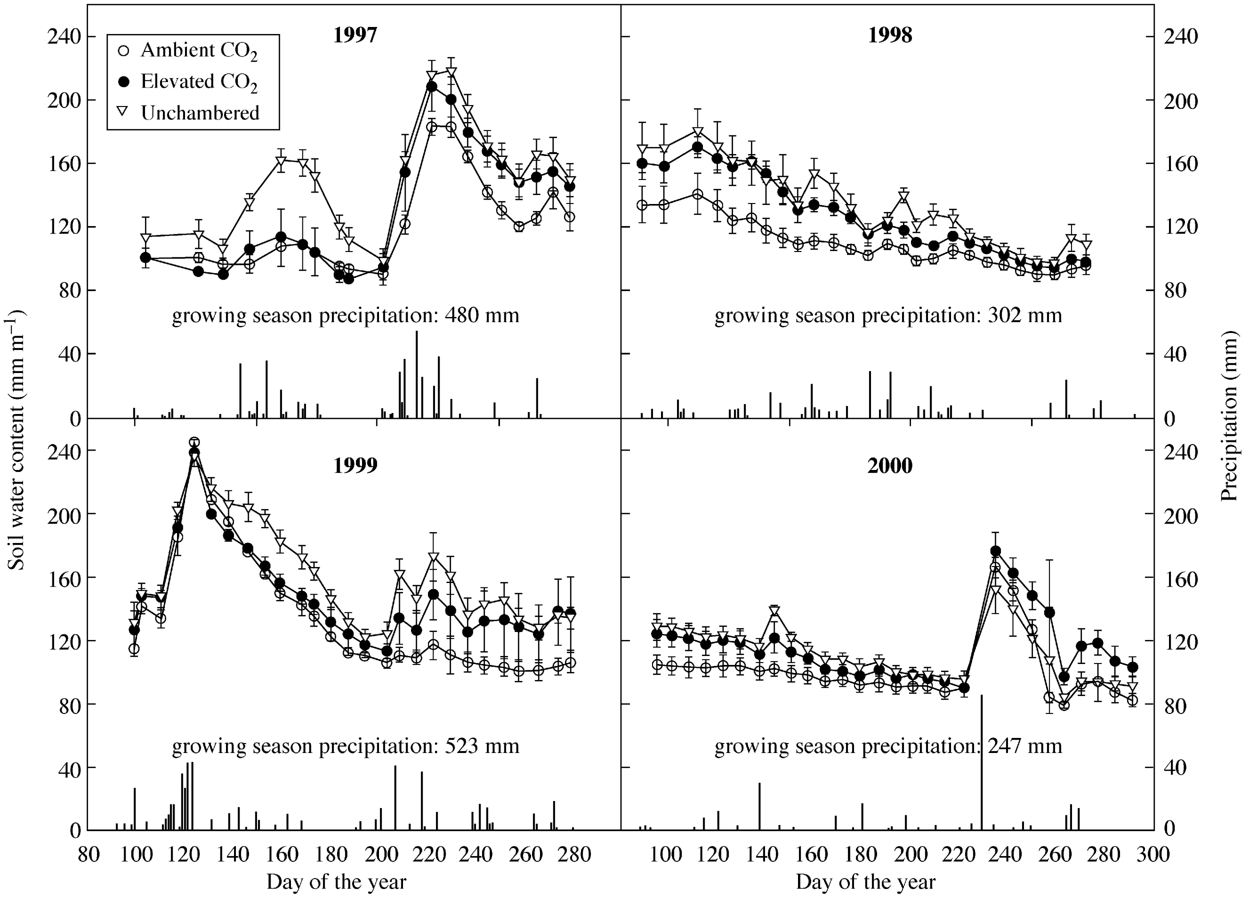

FIG. 1. Soil moisture (to 1 m) and growing season precipitation for 4 years in open-top chambers with ambient (360 µmol mol^{-1}) and elevated (720 µmol mol^{-1}) CO_2 and in plots without chambers on the Colorado shortgrass steppe. Data are means of three replications ± s.e.

Caemmerer *et al.*, 2001). Therefore, field studies suggest that the photosynthetic responses to elevated CO_2 in grasslands are site- and species-dependent. A deficit of resources other than CO_2 (soil N, soil water, rooting volume) can limit the production of new sink tissues when assimilation rates are improved (Drake *et al.*, 1997; Rogers *et al.*, 1998; Lee *et al.*, 2001). This source/sink imbalance causes an accumulation of carbohydrates, which is thought to feed back on photosynthetic processes (Sage, 1994; Stitt and Krapp, 1999).

Growth chamber studies have been used to investigate the photosynthetic and growth responses of a C_3 [Western wheatgrass; *Pascopyrum smithii* (Rydb.) A. Love] and a C_4 grass [Blue grama; *Bouteoloua gracilis* (H.B.K.) Lag.] to elevated CO_2 (Morgan *et al.*, 1994*a*, *b*; Hunt *et al.*, 1996; Read *et al.*, 1997; LeCain and Morgan, 1998; Morgan *et al.*, 1998). These are major species of the semi-arid shortgrass steppe (SGS) of the North American Great Plains (Milchunas *et al.*, 1989). Photosynthetic acclimation to elevated CO_2 was seen in both C_3 and C_4 species (Morgan *et al.*, 1994*a*; Read *et al.*, 1997; LeCain and Morgan, 1998). Despite the relative reduction, photosynthesis rates were slightly higher under elevated *vs.* ambient CO_2. Growth improvement in both species was attributed to higher rates of photosynthesis and improved soil and plant water status (Morgan *et al.*, 1994,*a*, *b*; Read and Morgan, 1996; Morgan *et al.*, 1998).

To increase understanding of the responses of C_3 and C_4 grasses to elevated CO_2 in a water-limited ecosystem, a field study was performed on the native SGS in north-eastern Colorado, USA. We hypothesized that photosynthesis of both *P. smithii* and *B. gracilis* would acclimate under elevated CO_2, but that there would still be a direct photosynthetic stimulation, especially in *P. smithii*. Leaf nitrogen, carbohydrates and assimilation at varying intercellular [CO_2] were measured to investigate the physiology of photosynthetic adaptations. We also hypothesized that stomatal conductance and transpiration of leaves would be reduced under elevated CO_2, and that stomatal acclimation would not occur. Measurements were conducted over four seasons (1997–2000) to document the responses to seasonal and CO_2-induced soil moisture variation.

MATERIALS AND METHODS

The study site is the USDA-ARS Central Plains Experimental Range, 40°50′N, 104°47′W, in the SGS region of north-eastern Colorado, USA. Mean annual

TABLE 1. *The maximum rate of carboxylation by Rubisco* ($V_{c,max}$), *the PPF-saturated rate of electron transport* (J_{max}), *assimilation at saturating light and* CO_2 (A_{sat}), *and the apparent quantum efficiency* (Q) *in* Pascopyrum smithii *(C_3) at three periods during the growing season*

	$V_{c,max}$ (μmol CO_2 m^{-2} s^{-1})	J_{max} (μmol e^- m^{-2} s^{-1})	A_{sat} (μmol CO_2 m^{-2} s^{-1})	Q (CO_2/photon)
Early season				
Ambient CO_2	63·3 ± 4	206 ± 20	32·8 ± 2·4	0·066 ± 0·01
Elevated CO_2	40·9 ± 4	121 ± 16	19·9 ± 2·4	0·076 ± 0·007
Mid-season				
Ambient CO_2	43·4 ± 9	97 ± 21	15·5 ± 3·7	0·051 ± 0·009
Elevated CO_2	28·2 ± 3	57 ± 7	10·1 ± 1·3	0·053 ± 0·008
Late season				
Ambient CO_2	62·9 ± 6	155 ± 19	24·5 ± 3·3	0·053 ± 0·013
Elevated CO_2	39·2 ± 5	99 ± 15	16·2 ± 3·4	0·053 ± 0·004
P				
CO_2trt	0·0001	0·0003	0·0006	0·4968
Year	0·8744	0·3395	0·2531	0·6905
Season	0·0132	0·0001	0·0001	0·1527
CO_2trt × year	0·7552	0·5467	0·6649	0·8450
CO_2trt × season	0·7006	0·4207	0·4028	0·9547

Plants were grown in open-top chambers on native shortgrass steppe of Colorado, USA, with ambient or elevated (360 and 720 μmol mol^{-1}) CO_2. Data are averaged across three years and three replications ± s.e.

TABLE 2. *The initial slope of the* A : C_i *curve (carboxylation efficiency,* CE*), assimilation at saturating light and* CO_2 (A_{sat}), *and the apparent quantum efficiency* (Q) *in* Bouteloua gracilis *(C_4) at three periods during the growing season*

	CE (CO_2 m^{-2} s^{-1}/mol^{-1} CO_2)	A_{sat} (μmol CO_2 m^{-2} s^{-1})	Q (CO_2/photon)
Early season			
Ambient CO_2	0·1267 ± 0·017	25·8 ± 3·7	0·047 ± 0·007
Elevated CO_2	0·1107 ± 0·11	22·8 ± 2·2	0·064 ± 0·002
Mid-season			
Ambient CO_2	0·0697 ± 0·07	12·7 ± 3·2	0·046 ± 0·007
Elevated CO_2	0·0726 ± 0·013	16·0 ± 2·6	0·052 ± 0·009
Late season			
Ambient CO_2	0·1325 ± 0·024	23·9 ± 3·9	0·052 ± 0·006
Elevated CO_2	0·1380 ± 0·021	25·3 ± 3·4	0·066 ± 0·007
P			
CO_2trt	0·8560	0·8948	0·2573
Year	0·2877	0·1075	0·6451
Season	0·0048	0·0029	0·1622
CO_2trt × year	0·9026	0·7447	0·6857
CO_2trt × season	0·8240	0·5861	0·5628

Plants were grown in open-top chambers on native shortgrass steppe of Colorado, USA, with ambient or elevated (360 and 720 μmol mol^{-1}) CO_2. Data are averaged across three years and three replications ± s.e.

precipitation (55 years) averaged 320 mm, and air temperatures averaged 15·6 °C in summer and 0·6 °C in winter, with maximum July temperatures of 30·6 °C (Milchunas *et al.*, 1989). The soil at the experimental site is a Remmit fine sandy loam (Ustollic camborthids) to greater than 1 m. This is considered to be a nitrogen-poor soil (Hunt *et al.*, 1988), with a total nitrogen content of 0·085 % in the upper 30 cm (A. Mosier, pers. comm.).

This experiment was established in spring 1997 on a 6 ha field of native grassland. Although much of the SGS is dominated by *B. gracilis* (Milchunas *et al.*, 1989), this site was chosen for its nearly equal mix of C_3 and C_4 grasses (Morgan *et al.*, 2001). The field was divided into three blocks, based on vegetation, and three plots per block were chosen at random. From mid-March until late October in 1997, 1998, 1999 and 2000, large (60·5 m^3) open-top chambers were placed on two plots in each of the three blocks. One chamber received ambient air (approx. 360 μmol mol^{-1} CO_2), the other received air with elevated CO_2 (720 μmol mol^{-1} CO_2). Each block also included an ‘unchambered control’ plot of equal ground area, which was used to monitor the effect of the chamber.

The chambers were 3·8 m high and 4·5 m in diameter, with plastic walls and top (Lexan; Regal Plastics, Littleton,

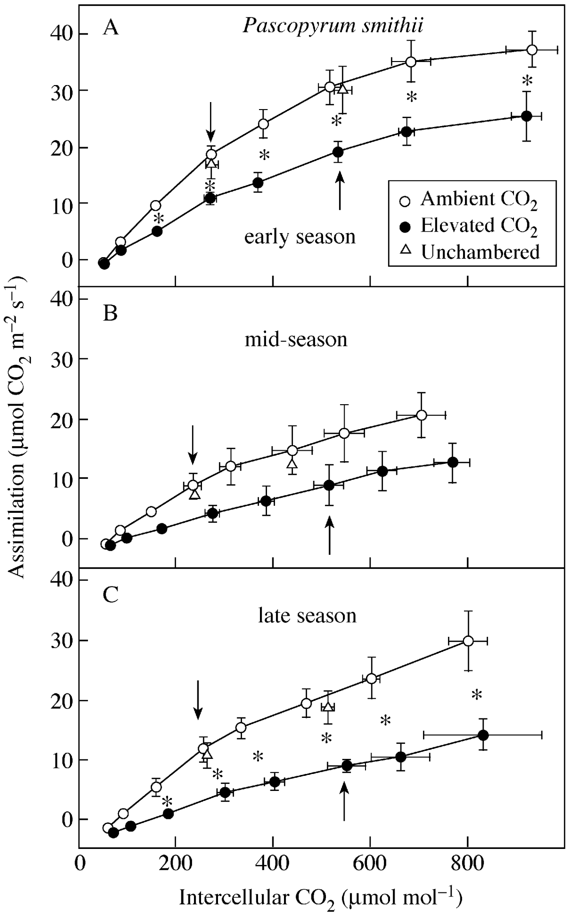

FIG. 2. Assimilation *vs.* intercellular CO_2 concentration of *Pascopyrum smithii* grown in open-top chambers with ambient (360 μmol mol^{-1}) and elevated (720 μmol mol^{-1}) CO_2, and in plots without chambers on the Colorado shortgrass steppe over the course of the 1999 growing season. Data are means of three replications ± s.e. An asterisk indicates a significant effect of CO_2 treatment at $P < 0{\cdot}05$. Arrows indicate data at the treatment [CO_2].

CO, USA), with a 0·75-m opening. An aluminium flange was buried to 0·75 m around the chamber wall. The chambers were aspirated at an exchange rate of 1·5 volumes min^{-1}. The CO_2 concentration was monitored using an infrared gas analyser (LI6262; LI-COR, Lincoln, NE, USA) and was elevated with pure CO_2 in three of the chambers (720 ± 20 μmol mol^{-1}). Air temperature (15 cm above the soil) and soil temperature (10 cm depth), measured using thermocouples, light intensity above the plant canopy (LI-190SA; LI-COR) and relative humidity at 2 m height (Vaisala HMP45C; Campbell Scientific, Logan, UT, USA) were recorded hourly. Since the chamber top kept out much of the precipitation, rain was captured and returned to the plots using an automatic system. For a more detailed description of the chambers see Morgan *et al.* (2001). Soil water content (SWC) was measured weekly using a neutron probe (model 4301; Troxler Electronics Lab., Research Triangle Park, NC, USA).

Exchange of CO_2 and H_2O were measured on recently expanded leaves of *B. gracilis* and *P. smithii* using a portable gas analysis system [CIRAS-1with PLC (N) leaf chamber; PP systems, Hitchin, UK]. Steady-state measurements of CO_2 assimilation rate (A), stomatal conductance to water vapour (g_s), transpiration (E) and intercellular CO_2 concentration (C_i) at cuvette concentrations (C_a) of 50, 100, 200, 360, 500, 720, 900 (and 1200 in *P. smithii*) μmol mol^{-1} CO_2 were made on leaves during mid-May, mid-July and late August (early, mid- and late season) in 1997, 1998 and 1999 in the ambient and elevated CO_2 plots. Drought prevented reliable $A : C_i$ measurement in 2000. Cuvette temperature and vapour pressure were set to ambient conditions and kept constant when varying C_a. Measurements were performed in the unchambered plots only at ambient CO_2.

Since responses to seasonal and CO_2-induced variation in soil water were of interest, A, g_s, E and the leaf water-use efficiency (WUE; CO_2 gain/H_2O loss) were measured at the two growth CO_2 concentrations every 3 to 4 weeks during 1997, 1998, 1999 and 2000 on all plots. Leaf water potential (ψ_{leaf}) was measured on these dates using a pressure chamber (Plant Measurement Systems, Corvalis, OR, USA). The light unit of the gas exchange system provided 1300 μmol m^{-2} s^{-1} photosynthetic photon flux (PPF). Photosynthesis was saturated at this light intensity in both species (data not shown). In addition, during early, mid- and late season, the photosynthetic response to varying light intensity was measured at the growth CO_2 concentration at 0, 50, 100, 160, 290, 590, 850 and 1300 μmol m^{-2} s^{-1} PPF.

Leaves similar to those in the cuvette were collected at midday, frozen in liquid nitrogen, lyophylized and ground to 0·5 mm. The content of starch, sucrose, hexose, fructans and total non-structural carbohydrates (TNC) was determined using a modified method of Hendrix (1993) (Morgan *et al.*, 1998). Nitrogen concentration was measured on a combustion N analyser (PDZ Europa Ltd, Northwich, UK). Leaf carbohydrates and N were calculated on a structural dry mass basis by subtracting TNC from total leaf dry mass.

The mechanistic analysis of $A : C_i$ and A : PPF curves for *P. smithii* (C_3) were made using 'Photosynthesis Assistant' software (Dundee Scientific, Dundee, UK). The maximum rate of carboxylation by ribulose-1,5 bisphosphate carboxylase/oxygenase (Rubisco) ($V_{c,max}$), the PPF-saturated rate of electron transport (J_{max}), and the apparent quantum efficiency (Q) were calculated. The light- and CO_2-saturated assimilation rate (A_{sat}) was also determined from $A : C_i$ curves. The mechanistic interpretation of $A : C_i$ curves is not well worked out for C_4 plants. Therefore, to investigate photosynthetic adaptations in *B. gracilis*, the carboxylation efficiency (CE), which is an indicator of efficiency of the phosphoenol-pyruvate carboxylase CO_2 pump, was calculated from the initial slope of the $A : C_i$ curves (Long *et al.*, 1993; Sage, 1994). A_{sat} and Q were also calculated for *B. gracilis*.

Data presented in the figures are means of three replications ± s.e., with statistical significance tested using the SAS ANOVA procedure (SAS Institute Inc., Cary, NC, USA). Data in the tables were analysed using the SAS PROC MIXED procedure, with year as a repeated measures variable. Correlations between parameters were

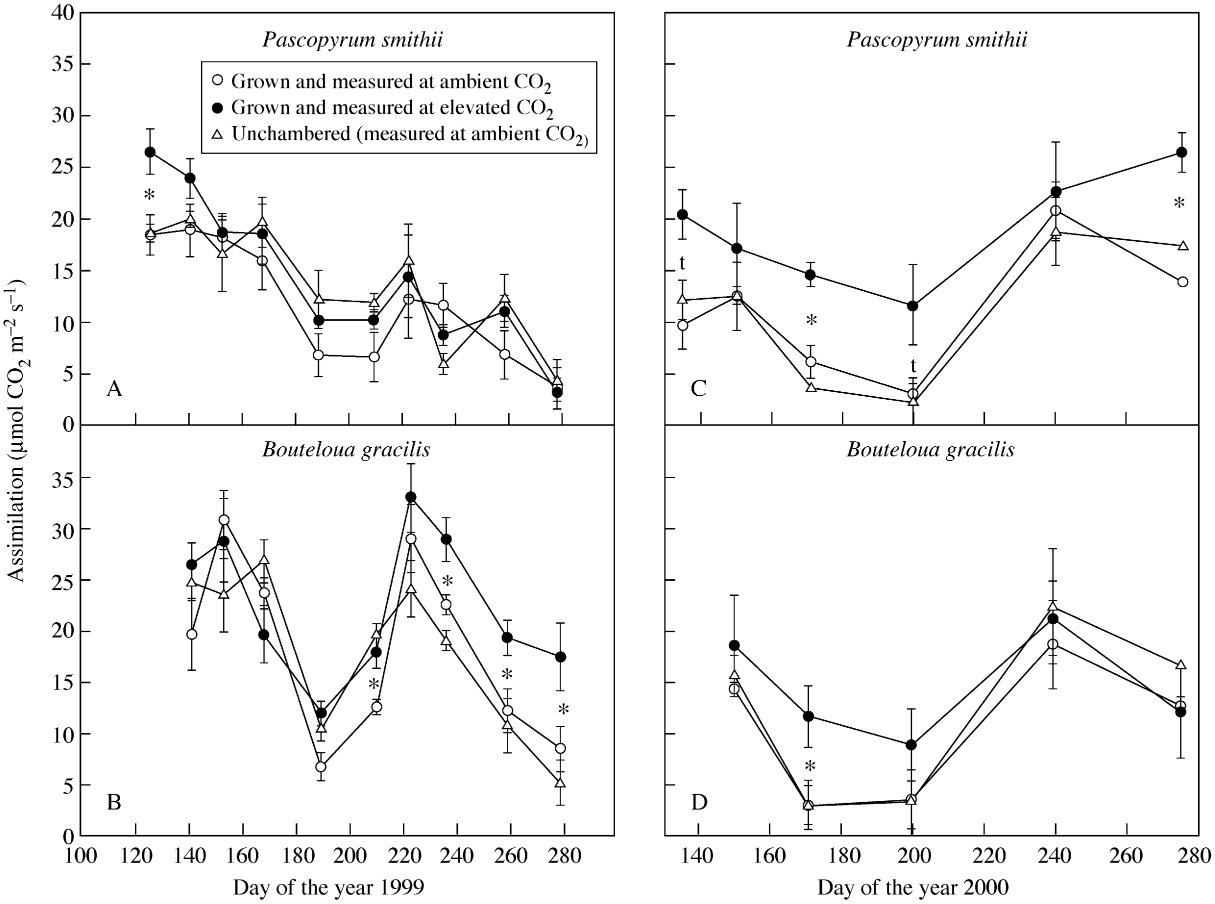

FIG. 3. Assimilation of *Pascopyrum smithii* and *Boutleoua gracilis* grown in open-top chambers with ambient (360 µmol mol^{-1}) and elevated (720 µmol mol^{-1}) CO_2, and in plots without chambers on the Colorado shortgrass steppe during the growing seasons of 1999 and 2000. Measurements were made at the chamber CO_2 concentrations. Data are means of three replications ± s.e. An asterisk indicates an effect of CO_2 treatment significant at P < 0·05; t, P < 0·10.

tested using the SAS PROC REG or PROC NLIN procedures, depending on the best fit of the data.

RESULTS

Chamber conditions, precipitation and soil water content

Air and soil temperatures in the chambers were, on average, 2·6 and 1·3 °C higher than in the unchambered plots, but there was no difference in vapour pressure. The plastic covering blocked approx. 5 % of the PPF, while shading from the chamber framework reduced daily PPF by 28 %.

Total growing season precipitation was 480, 302, 523 and 247 mm in 1997, 1998, 1999 and 2000 (long term average = 280 mm). Soil water content was higher in the elevated *vs.* ambient CO_2 chambers on many dates (Fig. 1). When averaged over the 4 years of study, SWC in the upper metre was 129·4 mm in elevated CO_2 chambers compared with 113·6 mm in ambient CO_2 chambers, an improvement of 14 % (P = 0·02). Ambient CO_2 chambers often had lower SWC than the unchambered plots owing to higher air temperatures.

Photosynthetic adaptations to elevated CO_2

Table 1 shows photosynthetic parameters for *P. smithii*. Long-term growth at elevated CO_2 reduced $V_{c,max}$, J_{max}, and A_{sat} by an average of 36, 39, and 36 % respectively (all P < 0·001). This indicates strong and consistent photosynthetic acclimation in this C_3 grass. Although there was a significant seasonal effect on these parameters, there was no interaction between CO_2 treatment and season or year.

The CO_2 effect on CE and A_{sat} was not significant in *B. gracilis* (Table 2). Again, there was a significant effect of season, probably due to seasonal differences in soil water (see below). However, the interaction between season and CO_2 treatment was again not significant.

There was no consistent effect of CO_2 on the light response curves of photosynthesis (measured at the growth CO_2 concentration) at any time or in either species (curves not shown). The apparent quantum efficiency (Q) is summarized in Tables 1 and 2, and shows no significant variable effect in either *P. smithii* or *B. gracilis*.

Since there was no CO_2 effect, further presentation of $A : C_i$ data from *B. gracilis* are not shown. The $A : C_i$ curves

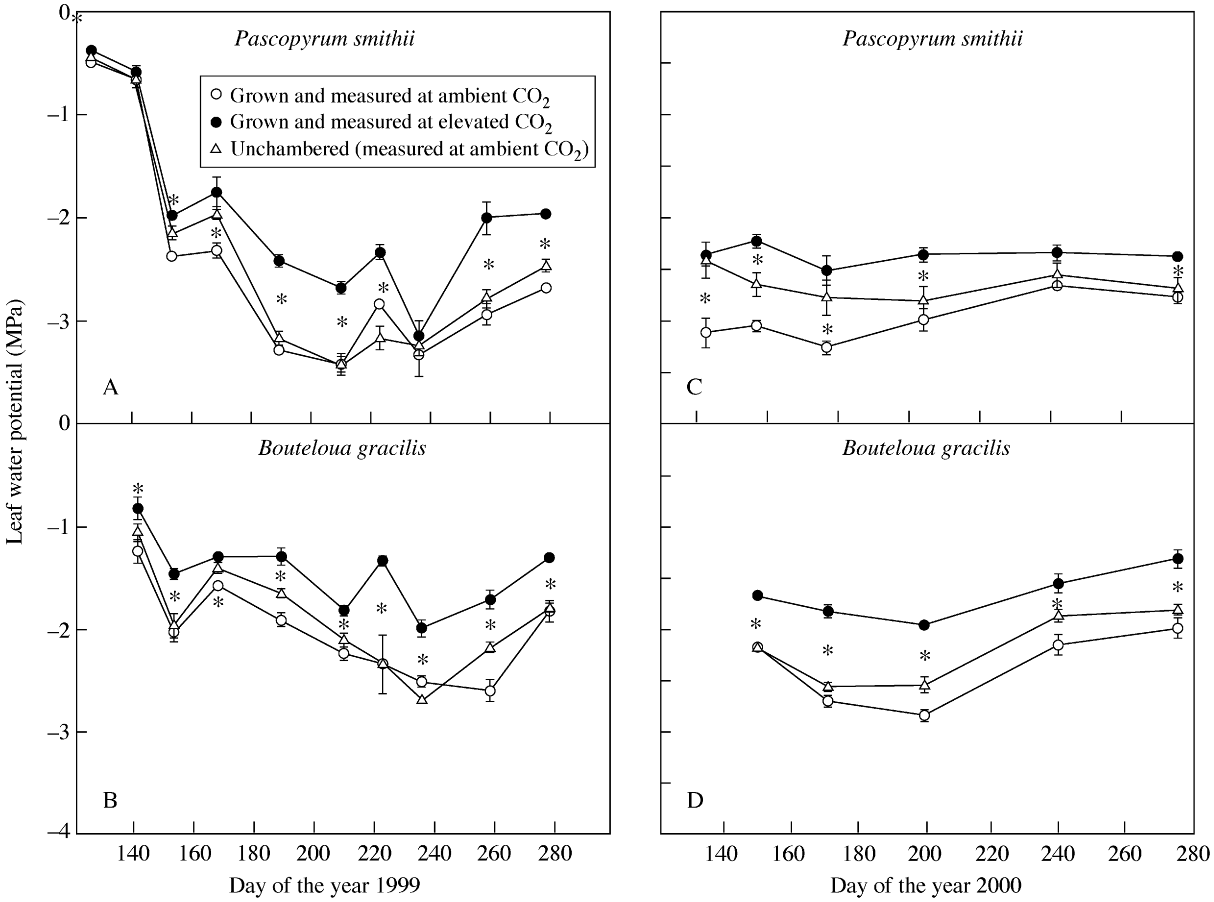

FIG. 4. Leaf water potential of *Pascopyrum smithii* and *Boutleoua gracilis* grown in open-top chambers with ambient (360 µmol mol^{-1}) and elevated (720 µmol mol^{-1}) CO_2, and in plots without chambers on the Colorado shortgrass steppe during the growing seasons of 1999 and 2000. Data are means of three replications ± s.e. An asterisk indicates a significant effect of CO_2 treatment at $P < 0{\cdot}05$.

of *P. smithii* from 1999 are given (Fig. 2) as this year was representative of the treatment responses. Early in the season, when SWC was high, *A* was reduced in elevated *vs.* ambient CO_2 leaves at most C_i (Fig. 2A). During mid-season, when plants were water-stressed, *A* was about 50 % of that measured in May and there was a (non-significant) trend for lower *A* in leaves grown at elevated CO_2 (Fig. 2B). Precipitation in late July improved *A*, and a significant reduction in the $A : C_i$ curve in leaves at elevated CO_2 was again seen late in the season (Fig. 2C). The reduction in photosynthetic capacity is such that *A* measured at the growth CO_2 concentration was similar in grasses grown at ambient and elevated CO_2 (Fig. 2, arrows). The assimilation rate in grasses in unchambered plots was similar to that in grasses in ambient CO_2 chambers.

Assimilation rates under seasonal and CO_2-induced soil moisture variation

Variability in the patterns of *A* measured at the growth CO_2 concentration was primarily a function of variation in SWC induced by season and CO_2. Here we contrast results from a wet (1999) and a dry (2000) year. Data for 1997 and 1998 have been presented previously (Morgan *et al.*, 2001).

Early in the season of 1999 [day of the year (DOY) 125], *P. smithii* plants grown at elevated CO_2 had higher *A* than plants grown at ambient CO_2 or without chambers (Fig. 3A). By DOY 153, this advantage had disappeared. However, as seasonal water stress developed, there were periods (DOY 188 and 210) when the assimilation rate of *P. smithii* grown at elevated CO_2 tended to be higher, probably due to better SWC in the elevated CO_2 plots (Fig. 1). For *B. gracili*, there was little difference in *A* early in the season of 1999 (Fig. 3B). However, during drought, and for the later third of the season, *A* of the elevated CO_2 plants was higher than that of ambient CO_2 plants (DOY 188, 210 and >225). Again, this was associated with improved SWC under elevated CO_2 (Fig. 1).

During 2000, elevated CO_2 plots had higher SWC for much of the season (Fig. 1). Assimilation rates of *P. smithii* reflect these soil moisture differences, with the elevated CO_2 plants having higher *A* than ambient plants on most dates (Fig. 3C). This effect was less evident in *B. gracilis* (Fig. 3D). Assimilation in the unchambered plots was similar to that in the ambient plots in both species, even though soil moisture was similar to that in elevated CO_2 plots (Fig. 1). This was associated with improved ψ_{leaf} in the elevated CO_2 plants.

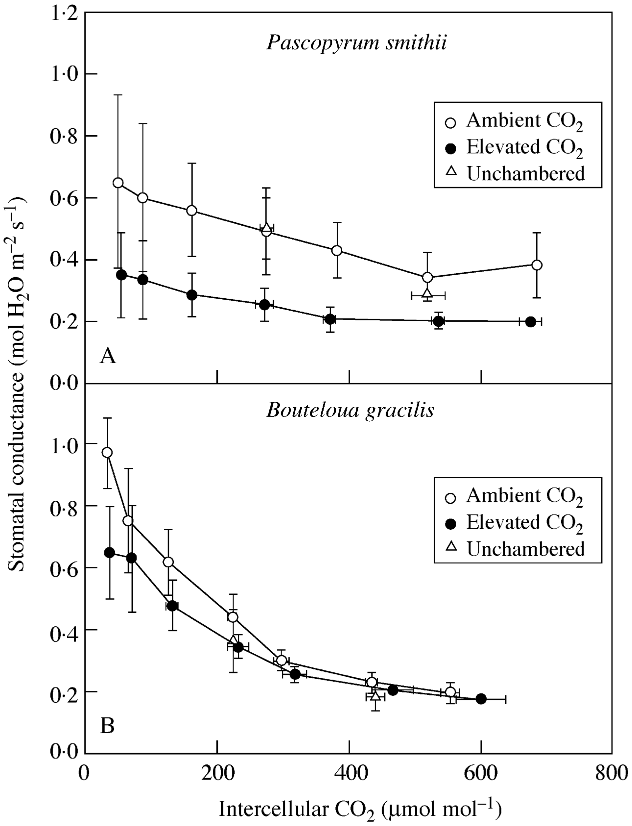

FIG. 5. Stomatal conductance *vs.* intercellular CO_2 of *Pascopyrum smithii* and *Boutleoua gracilis* grown in open-top chambers with ambient (360 µmol mol^{-1}) and elevated (720 µmol mol^{-1}) CO_2 and in plots without chambers on the Colorado shortgrass steppe. Measurements made during May 1999. Data are means of three replications ± s.e. The CO_2 treatment effect was not significant.

Leaf water potential under seasonal and CO_2*-induced soil moisture variation*

Higher SWC and improved conditions for *A* under elevated CO_2 were also reflected in improved ψ_{leaf}. Leaf water potential in both species was higher in plants grown at elevated CO_2 on most measurements dates in 1999 and 2000 (Fig. 4).

Stomatal responses to elevated CO_2

Responses of g_s to varying C_i early in the season of 1999 were representative of responses when interactions with drought were not occurring (see below) and thus are discussed here (Fig. 5). Stomatal conductance of *P. smithii* decreased from 0·5 to 0·28 mol m^{-2} s^{-1} over a range of C_i from 50 to 670 µmol mol^{-1}; this variation was less than that seen in *B. gracilis* which varied from 0·8 to 0·18 over similar values of C_i. In *P. smithii* there was a trend for lower g_s in leaves grown in elevated CO_2 (Fig. 5A). This trend was observed on most measurement dates. There was no indication of stomatal adaptation to elevated CO_2 in *B. gracilis* (Fig. 5B).

During much of the growing season of 1999, stomatal conductance of *P. smithii* (measured at the growth CO_2 concentration) was lower in plants grown at elevated CO_2 (Fig. 6A). Stomatal conductance of *B. gracilis* in elevated CO_2 chambers also tended to be lower, although the difference was significant on fewer dates (Fig. 6B). During the dry spring of 2000, g_s was sometimes higher in *P. smithii* plants from elevated CO_2, and there were no differences in g_s in *B. gracilis* (Fig. 6C and D). Leaf water-use efficiency of both species was always higher when measured at elevated CO_2 (data not shown).

Leaf nitrogen and carbohydrates responses to elevated CO_2

There was a significant CO_2 × season interaction for leaf N and TNC. Therefore, statistics are shown for each measurement period (Table 3). Early in the season, there was no CO_2 effect on the leaf N of *P. smithii*, but N was reduced under elevated CO_2 in mid- and late season. Leaf N was reduced in *B. gracilis* in elevated CO_2 in all three periods.

The content of non-structural carbohydrates was higher in *P. smithii* leaves grown in elevated *vs.* ambient CO_2 in all three periods (Table 3). An increase in all of the individual sugar fractions was found, but fructans and sucrose were the dominant carbohydrates in *P. smithii* (data not shown). In contrast, *B. gracilis* grown in elevated CO_2 had significantly more carbohydrates only early in the season (primarily starch). In both species, unchambered plants had TNC and N contents similar to those of plants in the ambient CO_2 chambers.

DISCUSSION

Reports of photosynthetic acclimation under elevated CO_2 have been considered the exception in field studies (Sage, 1994; Drake *et al.*, 1997). Contrary to this, our hypothesis that *P. smithii* would develop photosynthetic acclimation was confirmed in this study. Although there were periods, in early spring, that reflected a direct photosynthetic stimulation in elevated CO_2, 3 years of $A : C_i$ measurements showed consistent photosynthetic acclimation in *P. smithii*. These findings agree with previous results of growth chamber studies (Morgan *et al.*, 1994*a*, 1998; Read *et al.*, 1997), but the photosynthetic acclimation was greater in the field.

Previous field studies of CO_2 enrichment in grassland ecosystems show contrasting evidence for photosynthetic acclimation. In the tall grass prairie and a Texas grassland there was no photosynthetic acclimation in either C_3 or C_4 species (Nie *et al.*, 1992; Hamerlynck *et al.*, 1997; Anderson *et al.*, 2001). However, photosynthetic acclimation was reported in C_3 species in a New Zealand pasture (von Caemmerer *et al.*, 2001) and in several diverse grassland species in the Minnesota prairie (Lee *et al.*, 2001).

From these reports it is difficult to generalize about the basis of photosynthetic acclimation under elevated CO_2, as species, soils, climate and experimental conditions vary. However, it seems clear that the reduction in photosynthetic capacity helps to restore the balance between carbon assimilation, other resources (water, nutrients, rooting

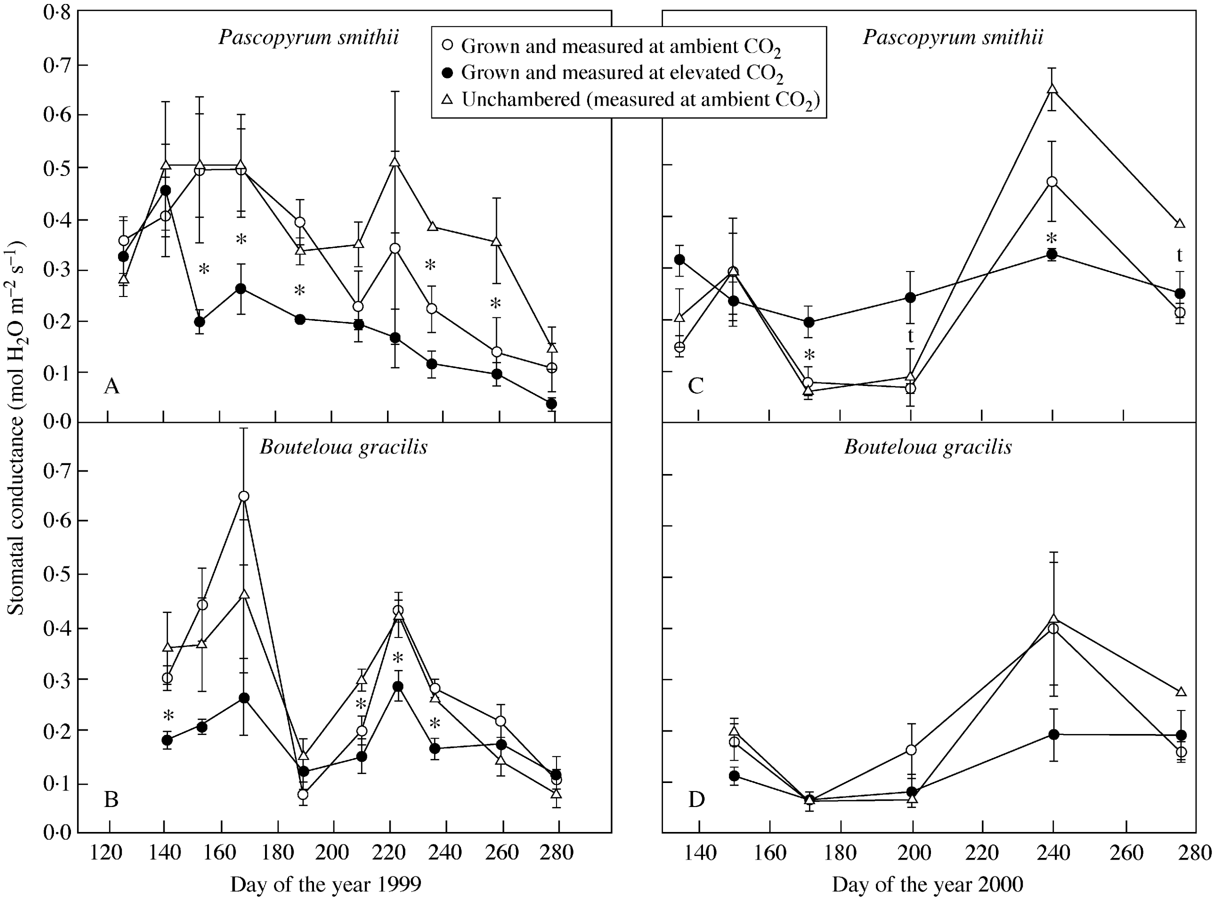

FIG. 6. Stomatal conductance of *Pascopyrum smithii* and *Boutleoua gracilis* grown in open-top chambers with ambient (360 µmol mol^{-1}) and elevated (720 µmol mol^{-1}) CO_2, and in plots without chambers on the Colorado shortgrass steppe during the growing seasons of 1999 and 2000. Measurements were made at the chamber CO_2 concentrations. Data are means of three replications ± s.e. An asterisk indicates an effect of CO_2 treatment significant at $P < 0{\cdot}05$; t, $P < 0{\cdot}10$.

volume) and carbon demand (Sage, 1994; Midgley *et al.*, 1999; Moore, 1999). In C_3 plants, this typically includes a reduction in Rubisco amount or activity, and reduced leaf N (Sage, 1994; Midgley *et al.*, 1999; Moore *et al.*, 1999; Stitt and Krapp, 1999).

Consistent with these studies, $V_{c,max}$ and leaf N concentration of *P. smithii* were significantly reduced under elevated CO_2. We suspect that improved growth of SGS species under elevated CO_2 (Morgan *et al.*, 2001) has further limited the available soil N in this N-limited ecosystem (Hunt *et al.*, 1988). The resultant inability of *P. smithii* plants to produce new sinks causes an imbalance in 'supply and demand', which feeds back on photosynthetic processes. Our leaf TNC data support this, as strong accumulation (average 61 %) of carbohydrates occurred. These results agree with those of Rogers *et al.* (1998) who concluded that photosynthetic acclimation results primarily from sink limitation in low N soils.

The $A : C_i$ curves obtained also show a reduction in the regeneration capacity for ribulose-1,5 bisphosphate (RuBP) in *P. smithii*, as indicated by reduced J_{max} (Table 1) (Long *et al.*, 1993). The light response data also support this (Table 1). The apparent quantum efficiency (Q) of photosynthesis is related to the RuBP regeneration capacity of C_3 plants (Long *et al.*, 1993). An increase in C_a should increase Q, owing to less oxygenation of RuBP. The lack of improvement in Q when measured at elevated CO_2 provides more evidence for a reduction in RuBP regeneration capacity (Table 1). These results agree with those of Midgley *et al.* (1999), who reported an integrated reduction in both carboxylation and RuBP regeneration capacity under elevated CO_2. However, these findings contradict the theory that Q will be improved in C_3 plants under increasing atmospheric CO_2 (Ehleringer *et al.*, 1997).

Contrary to our hypothesis, *B. gracilis* did not display photosynthetic acclimation under elevated CO_2, although we have previously reported acclimation of well-watered and fertilized *B. gracilis* (Read *et al.*, 1997; LeCain and Morgan, 1998). The $A : C_i$ curves in this field study show the short-term, 'potential' photosynthetic improvement at elevated CO_2 is only approx. 10 % in *B. gracilis* (data not shown), compared with approx. 65 % in *P. smithii*. Therefore, *B. gracilis* is less likely to experience source/sink imbalances. Compared with growth chamber studies,

TABLE 3. *Percentage nitrogen and total non-structural carbohydrates (TNC) on a structural dry mass basis of leaves of* Pascopyrum smithii *(C_3) and* Bouteloua gracilis *(C_4) grown in open-top chambers on native shortgrass steppe of Colorado, USA, with ambient or elevated (360 and 720 μmol mol^{-1}) CO_2, or on plots with no chamber*

	Nitrogen (%)		TNC (mg g^{-1})	
	P. smithii	*B. gracilis*	*P. smithii*	*B. gracilis*
Early season				
No chamber	1·59 ± 0·05	1·89 ± 0·03	111·6 ± 18·9	121·0 ± 10·9
Ambient CO_2	1·42 ± 0·03	1·65 ± 0·02	119·6 ± 6·6	110·9 ± 2·7
Elevated CO_2	1·33 ± 0·04	1·40 ± 0·05	193·1 ± 14·2	140·4 ± 11·3
P				
CO_2trt	0·1391	0·0001	0·0003	0·0103
Year	0·1106	0·0372	0·0001	0·0007
CO_2trt × year	0·1909	0·0723	0·1845	0·5604
Mid-season				
No chamber	1·33 ± 0·08	1·24 ± 0·06	111·0 ± 16·3	100·5 ± 1·2
Ambient CO_2	1·31 ± 0·05	1·15 ± 0·04	97·4 ± 7·9	106·7 ± 5·6
Elevated CO_2	1·10 ± 0·02	1·02 ± 0·02	176·5 ± 25·3	117·0 ± 5·8
P				
CO_2trt	0·0029	0·0013	0·0007	0·0868
Year	0·6238	0·0014	0·0310	0·0001
CO_2trt × year	0·9010	0·2490	0·1593	0·3753
Late season				
No chamber	1·05 ± 0·03	1·26 ± 0·04	72·9 ± 10·1	76·5 ± 3·0
Ambient CO_2	0·96 ± 0·06	1·20 ± 0·03	56·3 ± 7·1	74·7 ± 2·2
Elevated CO_2	0·77 ± 0·06	1·06 ± 0·02	83·5 ± 9·3	73·1 ± 6·2
P				
CO_2trt	0·0096	0·0250	0·0670	0·8580
Year	0·0001	0·0001	0·0001	0·0001
CO_2trt × year	0·3399	0·0029	0·0691	0·8497

Data are averaged over 4 years and three replications ± s.e. The season × CO_2 interaction was significant.
CO_2trt is the No chamber, ambient CO_2 and elevated CO_2 variables.

the low soil N availability in the field may have limited the potential photosynthetic response of *B. gracilis*, which precluded photosynthetic acclimation to elevated CO_2. Our results agree with those of von Caemmerer *et al.* (2001) who reported photosynthetic acclimation in C_3 species, but not C_4 species, under elevated CO_2. Due to acclimation, there was little improvement in *A* of *P. smithii* grown at elevated CO_2 during periods of adequate soil moisture. However, during dry periods, the improved soil moisture at elevated CO_2 resulted in improved *A* in both species. Averaged over the study, *A* was 30 and 21 % higher in *P. smithii* and *B. gracilis*, respectively, grown at elevated compared with ambient CO_2 (Table 4).

Figure 7 shows data for SWC and *A* in plants grown at elevated relative to ambient CO_2, plotted against the ambient CO_2 SWC, for 1999 and 2000. These plots demonstrate that the improvement in SWC under elevated CO_2 occurred primarily as soils dried out. Also, the improvement in *A* under elevated CO_2 was expressed primarily at low SWC. These results conflict with the views of Wand *et al.* (1999) that water stress reduces the relative photosynthetic enhancement of grasses grown at elevated CO_2. But our findings agree with those of Owensby *et al.* (1993) and Hamerlynck *et al.* (1997) for a tallgrass prairie, where responses were greater in drought seasons. Huxman *et al.* (1998), conducting a field study in the desert, also reported photosynthetic acclimation in *Larrea tridentata* Cav. when soil water was available, but assimilation was higher under elevated CO_2 during drought periods.

A reduction in g_s is an almost universal response to elevated CO_2 (Drake *et al.*, 1997; Jarvis *et al.*, 1999; Wand *et al.*, 1999). Consistent with our hypothesis, stomatal conductance was lower under elevated CO_2 compared with ambient CO_2 when soil moisture was adequate. Averaged over the study, stomatal conductance was reduced by 27 and 36 %, and transpiration was reduced 24 and 20 % in leaves of *P. smithii* and *B. gracilis* (Table 4). Reduced transpiration is probably the reason for improved SWC under elevated CO_2 (Field *et al.*, 1995).

An unexpected finding was that during periods in which the soil was very dry, g_s was sometimes higher in *P. smithii* grown under elevated CO_2 than that grown at ambient CO_2. The best example of this occurred in 2000, where higher SWC in elevated CO_2 plots was carried over from the previous year (Fig. 1). The lack of spring precipitation led to such extended drying of the soil that a point was reached where very low SWC under ambient CO_2 conditions actually reduced g_s below that of plants grown at elevated CO_2. Knapp *et al.* (1996) also reported an interaction between SWC and g_s during a dry year.

A consistent trend for lower g_s at an equivalent C_i was seen in *P. smithii* grown at elevated CO_2 (Fig. 5A). There is evidence that stomatal functioning is coupled with photosynthetic activity to maintain a relatively stable $C_i : C_a$ ratio

(Jarvis *et al.*, 1999). If stomata adapt to elevated CO_2 independently of photosynthetic adaptations, the $C_i : C_a$ ratio will differ (Ball and Berry, 1982). We found no effect of CO_2 treatment on $C_i : C_a$ in either species (data not shown). Our results agree with those of Drake *et al.* (1997) and Jarvis *et al.* (1999) that apparent stomatal acclimation is a result of stomatal dependence on photosynthetic processes.

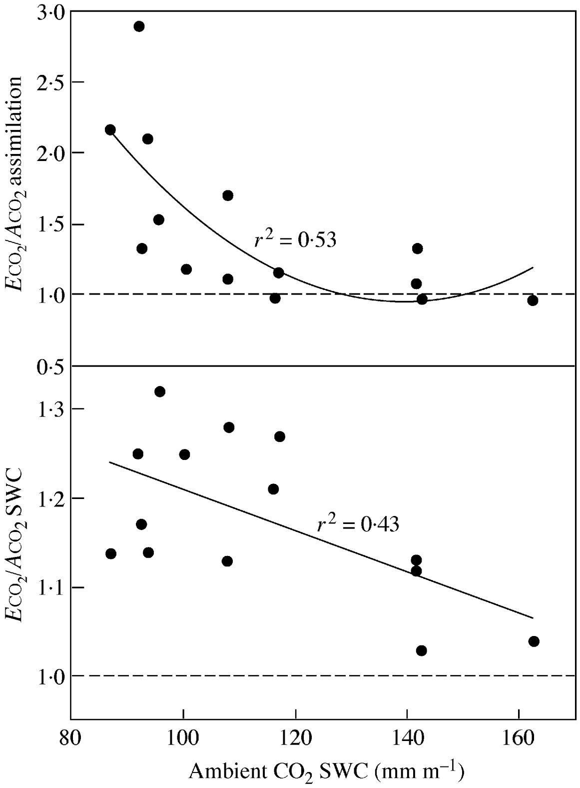

FIG. 7. 1999 and 2000 assimilation (*A*) and soil water content (SWC) data in elevated (E: 720 µmol mol^{-1}) relative to ambient (A: 360 µmol mol^{-1}) CO_2 chambers, plotted against the ambient CO_2 SWC. Lines are a linear regression for SWC and a second order polynomial for assimilation. Both correlations are significant ($P < 0·05$).

Leaf water potential was higher in elevated CO_2 plots on most dates. This is probably a result of improved SWC and g_s during much of the growing season (Tyree and Alexander, 1993; Hamerlynck *et al.*, 1997). However, the regression analysis showed poor correlations between the improvement in ψ_{leaf} and improved *A* and g_s (data not shown). This is because ψ_{leaf} was improved on nearly all dates, whereas *A* was improved primarily during periods of low SWC, and g_s interacted with SWC. Factors such as osmotic adjustment and rooting dynamics will complicate the relationship between ψ_{leaf} and *A* and g_s (Tyree and Alexander, 1993). Still, we believe that the strikingly consistent improvement in ψ_{leaf} is evidence that plant water status was more favourable for assimilation in the elevated CO_2 plots. The present results agree with those of Hamerlynck *et al.* (1997) who reported that elevated CO_2 improved ψ_{leaf} and *A* in both C_3 and C_4 species during drought years.

Implications for grassland ecosystems

In this semi-arid, N-limited ecosystem, the C_3 grass was unable to produce adequate new sinks to maintain higher *A* under elevated CO_2. Despite the lack of direct photosynthetic stimulation in both the C_3 and C_4 species, the present results provide support for a positive growth response to elevated CO_2 in this ecosystem. Averaging the gas exchange data over the study, *A* was improved by 30 and 21 %, g_s was reduced by 27 and 36 %, E was reduced by 24 and 20 %, and WUE was increased by 80 and 75 % in *P. smithii* and *B. gracilis* under elevated compared with ambient CO_2 (Table 4). A 26–47 % increase in above-ground biomass was found in the elevated CO_2 plots in 1997 and 1998 (Morgan *et al.*, 2001). In 1999, a year with nearly twice the average seasonal precipitation, biomass was improved by

TABLE 4. *Means of gas exchange data of* Pascopyrum smithii *and* Bouteloua gracilis *measured at the growth* CO_2 *concentration, and averaged over all of the data (4 years, 36 dates)*

	Assimilation (µmol CO_2 m^{-2} s^{-1})	Conductance (mol H_2O m^{-2} s^{-1})	Transpiration (mmol H_2O m^{-2} s^{-1})	Water-use efficiency (mmol CO_2/mol H_2O)
P. smithii				
Ambient CO_2	12·5	0·29	5·66	2·07
Elevated CO_2	16·2	0·21	4·32	3·76
P				
CO_2trt	0·0002	0·0038	0·0058	0·0001
Year	0·0330	0·0068	0·8393	0·0051
CO_2trt × year	0·4216	0·0681	0·0998	0·2757
B. gracilis				
Ambient CO_2	15·9	0·25	6·33	2·79
Elevated CO_2	19·3	0·16	5·16	4·89
P				
CO_2trt	0·0097	0·0001	0·0001	0·0001
Year	0·0008	0·0096	0·0035	0·2667
CO_2trt × year	0·9997	0·5184	0·9716	0·7657

Plants were grown in open-top chambers on native shortgrass steppe of Colorado, USA, with ambient or elevated (360 and 720 µmol mol^{-1}) CO_2.

only 17 % in elevated compared with ambient CO_2 (J. A. Morgan, pers. comm.). But during the drought year of 2000, biomass was increased by 95 % in elevated CO_2 plots. This large improvement in biomass was possible because of higher SWC and improved A and ψ_{leaf} in elevated CO_2 plots.

The present study suggests that improvements in WUE, soil water conservation and plant water relations will primarily affect responses to elevated CO_2 in this semi-arid ecosystem, rather than a direct effect on assimilation. Although the improvement in SWC (relative average 14 %) may seem small, the effect is large in this water-limited ecosystem. These results agree with those of Volk *et al.* (2000) that the effects of elevated CO_2 on soil moisture account for most of the variability in production. Improved soil moisture should extend the favourable growing season in arid and semi-arid ecosystems (Knapp *et al.*, 1996).

This study provides no evidence for a competitive advantage of the C_3 over the C_4 grass under elevated CO_2. Earlier notions that C_3 species would have a large advantage as global levels of CO_2 rise are not being supported by field studies (Owensby *et al.*, 1993; Wand *et al.*, 1999; Anderson *et al.*, 2001). The comparative response of C_3 *vs.* C_4 species will depend on phenology, morphology, root distribution, reproduction strategies and nutrient-use efficiency, as well as photosynthetic and stomatal responses.

ACKNOWLEDGEMENTS

The authors thank Scott Andre, Barry Weaver, Dean Ackerman and Dennis Mueller for designing and building the chambers, and for technical assistance, and Clenton Owensby, Jay Ham, Lisa Auen and Fred Caldwell of Kansas State University for guidance on chamber design. We also thank Alan Knapp and Lewis Ziska for critically reviewing the manuscript. This research was supported, in part, by funding from the NSF-TECO awards IBN-9524068 and USDA/NRICGP-98-134, NSF award DEB-9708596 and from the Shortgrass Steppe LTER project DEB 9350273. Mention of a trademark or manufacturer by the USDA does not imply its approval to the exclusion of other products or manufacturers that may also be suitable.

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