Global Change Biology (2002) **8,** 1118–1129

Soil- and plant-water dynamics in a C3/C4 grassland exposed to a subambient to superambient CO_2 gradient

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Abstract

Plants may be more sensitive to carbon dioxide (CO_2) enrichment at subambient concentrations than at superambient concentrations, but field tests are lacking. We measured soil-water content and determined xylem pressure potentials and $\delta^{13}C$ values of leaves of abundant species in a C3/C4 grassland exposed during 1997–1999 to a continuous gradient in atmospheric CO_2 spanning subambient through superambient concentrations (200–560 $\mu mol\ mol^{-1}$). We predicted that CO_2 enrichment would lessen soil-water depletion and increase xylem potentials more over subambient concentrations than over superambient concentrations. Because water-use efficiency of C3 species (net assimilation/leaf conductance; A/g) typically increases as soils dry, we hypothesized that improvements in plant-water relations at higher CO_2 would lessen positive effects of CO_2 enrichment on A/g. Depletion of soil water to 1.35 m depth was greater at low CO_2 concentrations than at higher CO_2 concentrations during a mid-season drought in 1998 and during late-season droughts in 1997 and 1999. During droughts each year, mid-day xylem potentials of the dominant C4 perennial grass (*Bothriochloa ischaemum* (L.) Keng) and the dominant C3 perennial forb (*Solanum dimidiatum* Raf.) became less negative as CO_2 increased from subambient to superambient concentrations. Leaf A/g—derived from leaf $\delta^{13}C$ values—was insensitive to feedbacks from CO_2 effects on soil water and plant water. Among most C3 species sampled—including annual grasses, perennial grasses and perennial forbs—A/g increased linearly with CO_2 across subambient concentrations. Leaf and air $\delta^{13}C$ values were too unstable at superambient CO_2 concentrations to reliably determine A/g. Significant changes in soil- and plant-water relations over subambient to superambient concentrations and in leaf A/g over subambient concentrations generally were not greater over low CO_2 than over higher CO_2. The continuous response of these variables to CO_2 suggests that atmospheric change has already improved water relations of grassland species and that periodically water-limited grasslands will remain sensitive to CO_2 enrichment.

Keywords: C3 species, C4 grasses, soil-water content, stable carbon isotopes, water-use efficiency, xylem potentials

Received 1 September 2001; revised version received 1 February 2002 and accepted 16 April 2002

Introduction

Improvements in plant-water relations are among the most influential effects of atmospheric carbon dioxide (CO_2) enrichment on water-limited ecosystems, including many grasslands (Jackson *et al*., 1994; Field *et al*., 1997; Owensby *et al*., 1997, 1999; Morgan *et al*., 2001). Much of the CO_2 benefit to water-limited plants derives from a decrease in stomatal conductance (Field *et al*., 1995; but see Pataki *et al*., 2000)—a reduction that may be greater over subambient concentrations characteristic of the past 420 000 years (Petit *et al*., 1999) than over superambient concentrations predicted for the future (Morison, 1987; Anderson *et al*., 2001; Maherali *et al*., 2002). Unless offset by greater leaf area or by atmospheric or other feedbacks (Field *et al*., 1995; Polley *et al*., 1997), lower conductance reduces transpiration rates, improves plant-water relations, increases plant water-use efficiency (Jackson *et al*., 1994; Hamerlynck *et al*., 1997; Owensby *et al*., 1997;

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Schapendonk *et al*., 1997) and may slow rates of soil-water depletion during drought (Fredeen *et al*., 1997; Owensby *et al*., 1997; Niklaus *et al*., 1998; Morgan *et al*., 2001).

In ecosystems that are at least periodically water-limited, CO_2-mediated changes in soil-water dynamics have been found to alter or even reverse physiological trends measured when water is plentiful. Knapp *et al*. (1996), for example, found that CO_2 enrichment consistently reduced stomatal conductance in tallgrass prairie when soils were wet. During drought, however, conductance actually was higher at elevated CO_2 than at ambient CO_2 for some species because soil-water content also was higher at elevated CO_2.

Shifts in water dynamics may similarly influence CO_2 effects on photosynthetic water-use efficiency (ratio of net assimilation rate to transpiration, $A/E = 1/\Delta w^* A/g$, where Δw is the mole fraction water vapour gradient from leaves to bulk air and g is the stomatal conductance to water vapour) and the closely related intrinsic water-use efficiency (ratio of net assimilation rate to stomatal conductance, A/g). Leaf A/g is positively related to the external CO_2 concentration (c_a) and negatively related to the ratio of leaf intercellular CO_2 concentration (c_i) to external CO_2 concentration (Polley *et al*., 1996) as:

$$A/g = c_a(1 - c_i/c_a)/1.6 \quad (1)$$

The A/g usually increases linearly and approximately proportionally with CO_2 in plants grown over both subambient (Polley *et al*., 1993) and superambient concentrations (Jackson *et al*., 1994), because the value of c_i/c_a is conserved in most species when water is plentiful (Sage, 1994; but see, Tissue *et al*., 1995). Both c_i/c_a and A/g are sensitive to soil and atmospheric drought, however. Leaf c_i/c_a usually declines and A/g increases when evaporative demand is high or soil water becomes limiting (Meinzer *et al*., 1990; Condon *et al*., 1992). By delaying soil-water depletion during drought, CO_2 enrichment may slow any decline in c_i/c_a and thereby limit drought-induced increases in leaf A/g.

Water dynamics have been studied in intact ecosystems only over ambient to superambient CO_2 concentrations. Consequently, little is known of how ecosystems responded as CO_2 concentration increased during the historical and prehistorical past. We measured soil-water content and determined xylem pressure potentials of leaves of abundant species in a C3/C4 grassland exposed for 1–3 years to a 200–560 $\mu mol\, mol^{-1}$ gradient in CO_2 concentration. We sought to test the prediction that CO_2 enrichment would lessen soil-water depletion and increase xylem potentials more over subambient concentrations than over superambient concentrations. We derived a temporally integrated measure of A/g for dominant C3 species exposed to subambient CO_2 concentrations by using c_i/c_a calculated from the difference between the stable carbon (C) isotope compositions ($\delta^{13}C$) of leaves of C3 plants (δ_p) and the CO_2 present in air (δ_a) in which leaves were grown (Δ) (Farquhar *et al*., 1982), where:

$$\Delta = (\delta_a - \delta_p)/(1 + \delta_p) = a + (b - a)c_i/c_a \quad (2)$$

and a and b are constants that describe isotopic fractionation resulting from the diffusion of CO_2 in air (4.4‰) and during carboxylation (27‰). We hypothesized that CO_2-mediated improvements in soil- and plant-water status would lessen CO_2 effects on A/g. Temperature and evaporative demand increase and soil-water availability typically declines from spring to summer in this system—changes that are associated in other systems with a seasonal decline in Δ and c_i/c_a and with an increase in A/g in C3 species (Smedley *et al*., 1991). We hypothesized, therefore, that Δ would decline from spring to summer in the grassland studied and that this decrease in Δ and the associated increase in A/g would be greater over low CO_2 concentrations than over CO_2 concentrations approaching the current level because of a greater seasonal decline in soil-water content and xylem potentials at subambient CO_2 concentrations.

Materials and methods

Carbon dioxide chambers/research site

We studied the effects of atmospheric CO_2 enrichment on a C3/C4 grassland in central Texas, USA (31°05′-N, 97°20′-W) with elongated field chambers that control CO_2 along continuous gradients from subambient to superambient concentrations (Johnson *et al*., 2000). The CO_2 facility consists of two transparent, tunnel-shaped chambers—each with 10 consecutive compartments that are 1 m wide and tall and 5 m long. Pure CO_2 is injected into one chamber during daylight in order to initiate a superambient CO_2 gradient (560–350 $\mu mol\, mol^{-1}$). Ambient air is introduced to the second chamber in order to initiate a subambient CO_2 gradient (365–200 $\mu mol\, mol^{-1}$). Nighttime CO_2 concentrations are regulated at about 150 $\mu mol\, mol^{-1}$ above daytime values along each chamber. Desired CO_2 concentration gradients are maintained by automatically varying the direction (daylight, night) and rate of airflow through chambers in response to changes in photosynthetic (daylight) or respiration rates (night). Increases in air temperature and water vapour that occur along chambers during daylight are suppressed by cooling and dehumidifying air at 5 m intervals, with the aim of regulating mean air temperature and vapour pressure deficit (vpd) in each compartment near ambient values. Soil beneath the chambers is separated from surrounding soil to a depth of 0.9 m with a rubber-coated fabric.

A continuous gradient in CO_2 from 560 to 200 μmol mol^{-1} was maintained on this grassland dominated by the C4 perennial grass *Bothriochloa ischaemum* (L.) Keng and the C3 perennial forbs *Solanum dimidiatum* Raf. and *Ratibida columnaris* (Sims) D. Don during growing seasons (March–November) of 1997–2000. Johnson *et al*. (2000) described, in detail, the regulation of CO_2 concentration and environmental parameters along chambers. Consistent CO_2 concentrations were maintained along chambers despite seasonal and annual variations in plant and environmental variables. During the 1998 growing season, for example, the standard error of daytime CO_2 concentration at each location sampled along chambers (calculated daily) varied from a mean of 0.9–2.7 at the air entrance of chambers to 2.1–4.8 μmol mol^{-1} at the air exit of chambers. Air temperatures were similar in subambient and in superambient chambers, but generally were 2–4 °C cooler than outside during the warmest period of the year (May–September). The vpd during daylight was lower, on average, in chambers than in surrounding grassland and, during 1998, was lower in the superambient chamber than in the subambient chamber only during the summer months of June–August (mean = 1.42 and 2.02 kPa, respectively). During the other months, vpd was not related to CO_2 concentration (not shown).

The annual precipitation at the research site averages 877 mm (87 years record). Rainfall was considerably lower than the average during substantial periods of each of the three years of this study. Droughts occurred late in the 1997 and 1999 growing seasons and during mid-season in 1998. Precipitation during the 3 months period of August–October 1997, for example, was 47% of the 87 years average for this period (224 mm). Precipitation during the 6 months period (March –August) of the 1998 growing season was 45% of the long-term mean for these months (448 mm). Most of 1999 was much drier than the average. Drought was particularly severe late in the 1999 growing season (August–November) when rainfall was only 26% of the average.

Measurement and control of soil water

Volumetric soil-water content to 1.35 m depth was measured weekly in the centre of each 5 m compartment of chambers with a neutron probe. In June of 1998, measurements of soil-water content were initiated in *Bothriochloa*-dominated grassland located 20 m to the west of CO_2 chambers. Soil-water content to 1.35 m depth was evaluated every 10 m along a 1-m-wide and 50-m-long transect that served as an unchambered control for the CO_2 experiment. Neutron attenuation was measured at 0.15-m-depth increments.

We also measured soil-water content in chamber compartments during 1996 prior to the imposition of CO_2 treatments. Water content to 1.35 m depth was determined at approximately 2 weeks intervals beginning in mid-July of 1996. Mean water content to 1.35 m depth increased from 29 cm in mid-July to 47.8 cm in late-December of 1996, but on no date in 1996 was soil water related to subsequent CO_2 treatment ($P = 0.16$–0.89).

Irrigation in the amount of rainfall was applied to the chambered grassland on the day following precipitation through July 1999. To better approximate the effects of rainfall on soil-water conditions in surrounding grassland, the irrigation regime was altered in August 1999. Subsequently, the amount of water applied to the entire system was determined weekly by subtracting the water content of soil in the chamber compartment maintained at 360 μmol mol^{-1} from the soil-water content of the surrounding grassland.

Xylem pressure potentials

During fall 1997 and throughout most of the 1998 and 1999 growing seasons, we measured xylem pressure potentials on the leaves (or the blades) of the dominant grass (*Bothriochloa*) and of the dominant forb (*Solanum*) from along the CO_2 gradient with a pressure chamber (Model 3005, Soil Moisture Equipment, Golita, CA, USA). Leaves were accessed through zippered openings in the polyethylene covering of each 5 m compartment along chambers, excised and immediately placed within the pressure chamber. Xylem potential was measured about every 2 weeks at mid-day (Ψ_m; 1100–1400 Central Standard Time) and monthly at predawn (Ψ_p) on two leaves per species from each 5 m compartment.

Sampling for C-isotope composition

Recently expanded and upper canopy leaves of the dominant C4 grass (*Bothriochloa*) and of the abundant C3 species (including annual and perennial grasses and perennial forbs) were collected from along the CO_2 gradient in May and September of 1998 and 1999 and in April of 2000 for measurements of $\delta^{13}C$. Leaves were collected from 1 m^2 areas near the beginning and end of each 5-m-long compartment. When possible, leaves were collected from multiple individuals per species and were composited by species for each 1 m^2 area sampled. Air samples were collected in duplicate in 1 L flasks from near the beginning, centre and end of both the superambient and subambient gradients in May of 1998 and 1999 for measurements of $\delta^{13}C$. The C-isotope composition of whole leaves (Isotope Services, Inc., Los Alamos, New Mexico, USA) and of CO_2 in air (Stable Isotope Laboratory, University of Colorado, Campas Box 450, Boulder, Colorado, USA) were determined by mass spectrometry and expressed as $\delta^{13}C$, ‰ (parts per thousand) ^{13}C relative to a PeeDee belemnite reference standard.

Photosynthesis progressively depletes the CO_2 concentration and increases the $^{13}C/^{12}C$ of air as it moves from the air intake to outlet of chambers (Polley *et al*., 1993). We measured the change in air $\delta^{13}C$ along the CO_2 gradient in May of 1998 and 1999 and used $\delta^{13}C$ values of *Bothriochloa* leaves (C4 grass) from along the CO_2 gradient as a proxy for air $\delta^{13}C$ at other sampling dates. The difference between the $\delta^{13}C$ of atmospheric CO_2 and leaf carbon of *Bothriochloa* was conserved across subambient CO_2 treatments and years, as it often is in C4 species (Polley *et al*., 1993). Slopes of linear regressions of $\delta^{13}C$ values for air and the C4 grass *Bothriochloa* on CO_2 concentration did not differ significantly in May of 1998 or of 1999 (lines were parallel, $P > 0.10$ in each year) over subambient to ambient CO_2 concentrations. Fractionation by *Bothriochloa* averaged 3.61‰ (3.66‰ and 3.56‰ in May of 1998 and of 1999, respectively). Over superambient concentrations, however, relationships between leaf and air $\delta^{13}C$ values and CO_2 usually were weak or were not significant. Air $\delta^{13}C$ values along the superambient gradient may have varied as injection rates of ^{13}C-depleted CO_2 were altered to match the changes in the rate at which air was introduced to the chamber or because of tank-to-tank variation in the $\delta^{13}C$ value of CO_2 that was injected. Consequently, we calculated isotopic discrimination (Δ) of C3 species over subambient to ambient CO_2 concentrations only.

By using the mean fractionation of 3.61‰ by *Bothriochloa*, we calculated the $\delta^{13}C$ of air in September of each year and in April of 2000 as a function of CO_2 concentration from leaf $\delta^{13}C$ values of the C4 grass. We used $\delta^{13}C$ measurements of air collected in May of each year and values of air $\delta^{13}C$ calculated from the C4 proxy at other harvests in order to calculate Δ by C3 species. From these values of Δ, we calculated temporally integrated values of c_i/c_a and of A/g (Eqns 1 and 2).

Statistics

The relationship between CO_2 concentration during daylight and distance along both superambient and subambient chambers was slightly curvilinear (Johnson *et al*., 2000). From these relationships, we calculated mean CO_2 concentrations for each meter and 5 m compartment along chambers. These CO_2 concentrations were used as the independent variable in regression analyses with C-isotope composition, soil-water content and xylem pressure potentials of leaves as dependent variables ($P < 0.05$ significance level). Linear, hyperbolic, power and logarithmic functions were fitted to data. The model with the greatest r^2 value was deemed the best fit. Seasonal changes in discrimination (Δ) of individual species were assessed with analysis of variance (ANOVA) when CO_2 did not affect Δ. Species differences in mean Δ were analysed with single degree of freedom contrasts.

Results

Xylem potentials

Seasonal changes in predawn (Ψ_p) and mid-day xylem potentials (Ψ_m) of dominant species mirrored seasonal trends in precipitation and soil-water content (Fig. 1). During 1998, for example, Ψ_p and Ψ_m of both *Bothriochloa* and *Solanum* reached the minimum values in August and September (Day of Year 213–273) following a 6 months drought. Both Ψ_p and Ψ_m of the grass and of the forb declined during the late-season drought in 1999.

For both *Bothriochloa* and *Solanum*, Ψ_m usually was less negative when averaged over superambient than over subambient CO_2 concentrations (Fig. 1). This increase in Ψ_m at higher CO_2 concentrations, however, was much more consistent in the grass than in the forb. During the latter part of 1997 and over most of the 1998 and 1999 growing seasons, Ψ_m of *Bothriochloa* increased significantly with CO_2 across subambient to superambient concentrations (Table 1). Carbon dioxide enrichment improved Ψ_m of *Solanum* most consistently during the latter part of the 1998 season.

For *Bothriochloa*, significant relationships between Ψ_m and CO_2 frequently became curvilinear, with greater increase over subambient concentrations than over superambient concentrations (Fig. 1, Table 1), near the end of the late-season droughts in 1997 and 1999 (~DOY 250–300) and near the end of the mid-season drought in 1998 (~DOY 250). In 1999, for example, relationships between CO_2 and Ψ_m of *Bothriochloa* generally were best described by linear regressions during early to mid-phases of the late-season drought, but became slightly curvilinear (Fig. 2) as drought persisted and soil-water content and Ψ_m declined (Fig. 1).

During the three years of sampling, CO_2 concentration had no consistent effect on Ψ_p of either *Bothriochloa* or of *Solanum* (Fig. 1). When Ψ_p was significantly related to CO_2 treatment, the trend often was the opposite of that expected, with Ψ_p decreasing slightly with increasing CO_2 concentration (not shown).

Carbon isotopes

The $\delta^{13}C$ values of air and of leaves of the C3 species and of the C4 grass *Bothriochloa* were linearly correlated with

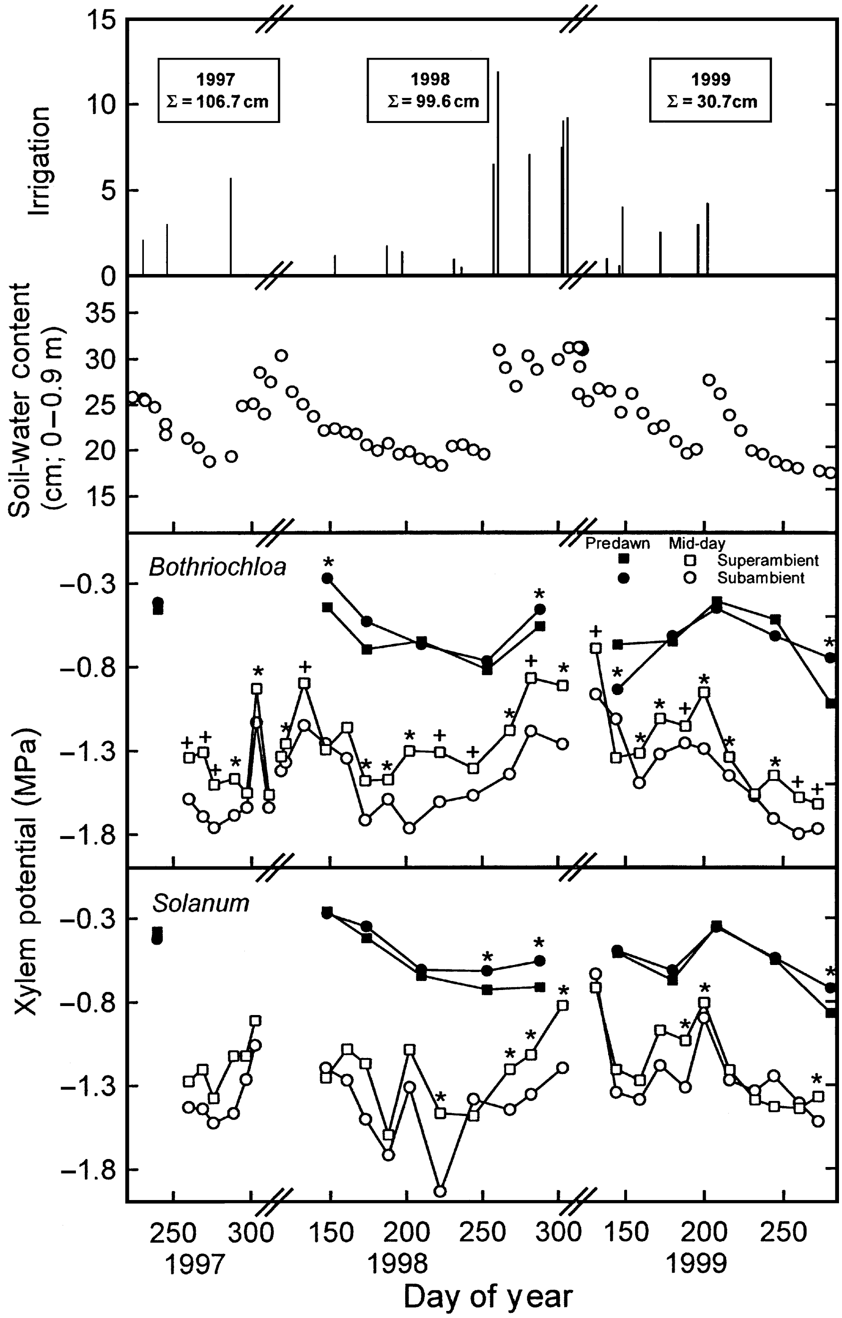

Fig. 1 Irrigation, soil-water content to 0.9 m depth at the current CO_2 concentration (mean = 360 μmol mol^{-1}), and predawn xylem potentials (Ψ_p) and mid-day xylem potentials (Ψ_m) of leaves of the dominant grass (*Bothriochloa*) and the dominant forb (*Solanum*) in grassland exposed in elongated chambers to a continuous gradient in CO_2 concentration. Insets in the upper panel denote the total water addition during each calendar year. Xylem potentials shown for each sampling date are averages over subambient (200–365 μmol mol^{-1}) and superambient CO_2 concentrations (350–560 μmol mol^{-1}). Significant ($P < 0.05$) linear relationships between Ψ_p or Ψ_m and CO_2 at subambient to superambient concentrations on a given measurement date are denoted with an asterisk (*). Significant curvilinear relationships (fit with hyperbolic or power functions) between Ψ_m and CO_2 are denoted by a plus (+). See Table 1 for results of regression analyses of Ψ_m on CO_2.

CO_2 over subambient concentrations at each sampling period (Fig. 3). Photosynthesis by plants enclosed in elongated chambers was used in order to maintain CO_2 gradients; consequently, the $\delta^{13}C$ values of air became less negative as CO_2 declined. Because discrimination against ^{13}C and the resulting increase in $^{13}C/^{12}C$ of the remaining CO_2 in air is greater during C fixation by C3 than by C4 species, seasonal changes in slopes of

Table 1 Results of regression analyses for significant relationships between mid-day xylem potentials (Ψ_m) of leaves of the C4 grass *Bothriochloa* and C3 forb *Solanum* and CO_2 concentration over subambient to superambient concentrations. Linear ($y = ax + b$), hyperbolic [$y = ax/(b + x)$], or power [$y = ax^b$] functions were fit to relationships of Ψ_m (y; MPa) to CO_2 concentration (x; μmol mol^{-1}) on each measurement date ($n = 29$–38)

Species, year and day of year	Model type	Slope or a-value	Intercept or b-value	r^2	*P*-value
Bothriochloa ischaemum					
1997					
DOY 260	Hyperbolic	−1.06	−91.74	0.58	< 0.0001
269	Hyperbolic	−1.04	−104.86	0.57	< 0.0001
276	Hyperbolic	−1.25	−80.61	0.53	< 0.0001
289	Linear	0.0008	−1.89	0.10	0.06
303	Linear	0.0014	−1.58	0.39	< 0.0001
1998					
DOY 121	Linear	0.0006	−1.54	0.24	0.002
133	Hyperbolic	−0.63	−135.15	0.58	< 0.0001
173	Linear	0.0011	−2.02	0.41	< 0.0001
188	Linear	0.0006	−1.76	0.14	0.02
202	Linear	0.0021	−2.33	0.39	< 0.0001
222	Hyperbolic	−1.02	−103.26	0.41	< 0.0001
244	Hyperbolic	−1.21	−64.32	0.18	0.008
268	Linear	0.0013	−1.82	0.25	0.002
282	Power	−80.78	−0.74	0.56	< 0.0001
303	Linear	0.0018	−1.79	0.56	< 0.0001
1999					
DOY 131	Hyperbolic	−0.49	−131.29	0.37	< 0.0001
159	Linear	0.0014	−1.96	0.21	0.007
172	Linear	0.0014	−1.75	0.30	0.005
188	Hyperbolic	−0.97	−81.84	0.25	0.004
200	Linear	0.0017	−1.78	0.48	< 0.0001
216	Linear	0.0007	−1.66	0.29	0.0006
244	Linear	0.0017	−2.24	0.60	< 0.0001
260	Hyperbolic	−1.35	−68.73	0.48	< 0.0001
272	Hyperbolic	−1.26	−87.98	0.30	0.0003
Solanum dimidiatum					
1998					
DOY 222	Linear	0.0016	−2.32	0.24	0.005
268	Linear	0.0011	−1.76	0.25	0.003
282	Linear	0.0010	−1.61	0.19	0.012
303	Linear	0.0015	−1.59	0.74	< 0.0001
1999					
DOY 188	Linear	0.0007	−1.50	0.20	0.01
200	Linear	0.0009	−1.39	0.27	0.004
272	Linear	0.0009	−1.71	0.24	0.015

relationships of leaf δ^{13}C with CO_2 reflect a seasonal shift in the relative contribution of C3 and C4 plants to CO_2 depletion. In both 1998 and 1999, δ^{13}C values of *Bothriochloa* leaves—which served as a proxy for air δ^{13}C values—increased less per unit decline in CO_2 concentration in September (2.8‰ and 3.5‰ per 100 μmol mol^{-1} decline in CO_2 in 1998 and 1999) than in May (4.1‰ and 4.5‰ per 100 μmol mol^{-1} decline in CO_2 in 1998 and 1999), reflecting a spring (May) to autumn (September) shift during each year toward greater C4 photosynthesis.

In only one C3 species (*Ambrosia*) did Δ, the difference between the δ^{13}C of atmospheric CO_2 and leaf C (Eqn. 2) change consistently with CO_2 concentration (Table 2). Discrimination by this perennial forb increased linearly with CO_2 by an average of 1.8 and 0.7‰ for each 100 μmol mol^{-1} increase in concentration in May and September of 1998. Because this species was relatively

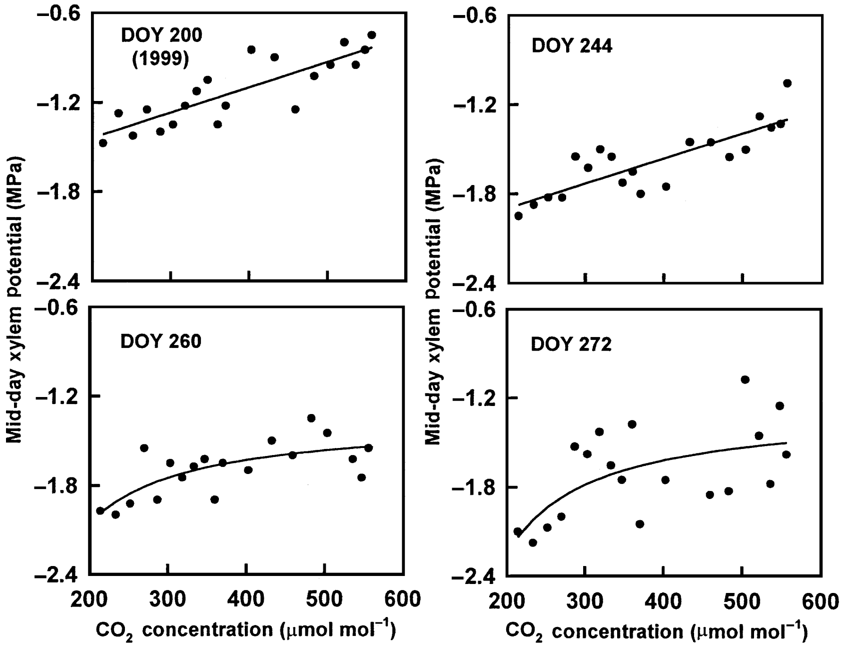

Fig. 2 Relationships between mid-day xylem potentials (Ψ_m) of leaves of the C4 grass *Bothriochloa* and daytime CO_2 concentration during a drought interval in 1999 that spanned DOY 200 through 272. Plotted are mean values of Ψ_m at each CO_2 concentration, but lines were fit with linear (DOY 200, 244) or hyperbolic (DOY 260, 272) functions by using all measurements ($n = 34$–38; see Table 1 for regression parameters).

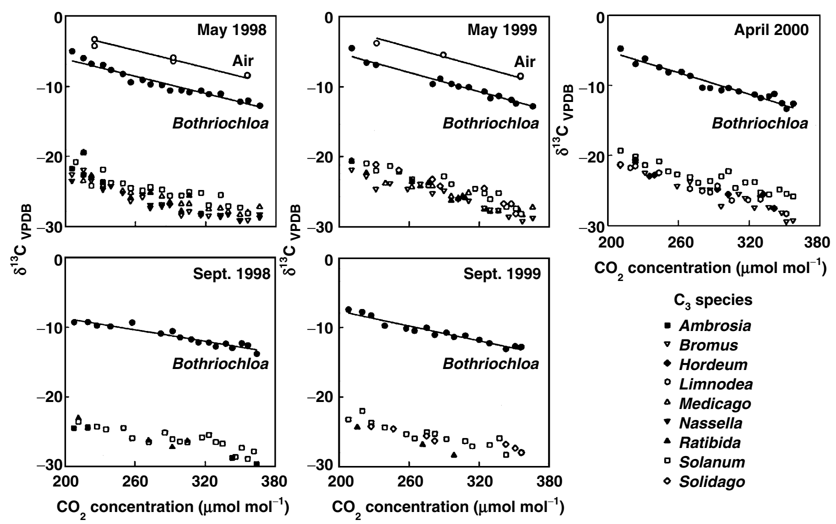

Fig. 3 The stable carbon isotope composition ($\delta^{13}C$) of air and of upper leaves of grassland species growing during May and September of 1998, May and September of 1999, and April of 2000 along a continuous gradient in CO_2 over subambient concentrations. Lines were fit by linear regression to relationships between $\delta^{13}C$ values of air and of the C4 species *Bothriochloa ischaemum* and CO_2 concentration. The C3 species sampled included annual grasses (*Bromus japonicus, Hordeum pusillum, Limnodea arkansana*), a perennial grass (*Nassella leucotricha*) and perennial forbs (*Ambrosia confertiflora, Medicago lupulina, Ratibida columnaris, Solanum dimidiatum, Solidago canadensis*).

rare, regressions were developed from only five samples in May and four samples in September. There was a quadratic relationship between Δ and CO_2 for *Bromus* in May 1998 (not shown), with maximum values of Δ at intermediate CO_2 concentrations. Discrimination declined linearly with increasing CO_2 concentration in *Medicago* in May 1998, in *Solidago* in September 1999 and in *Solanum* in April 2000, but there was a significant relationship between Δ and CO_2 concentration in none of these species at other harvests .

The absence of consistent changes in Δ across subambient CO_2 concentrations indicates that c_i/c_a values of the C3 species sampled were conserved (Eqn. 2) and that a given proportional increase in CO_2 concentration elicited a similar proportional increase in A/g (Eqn. 1). We calculated from regressions of A/g on CO_2 concentration for the five C3 species sampled in May 1999 that A/g increased by between 23 and 35% with a 33% increase in CO_2 from 270 to 360 μmol mol^{-1} (Fig. 4). Absolute increases in A/g over preindustrial to current CO_2 concentrations ranged between 14.4 μmol (CO_2) mol (H_2O)$^{-1}$ (*Solidago*) and 20.6 μmol (CO_2) mol (H_2O)$^{-1}$ (*Solanum*) for the four species sampled over this CO_2 range (excluding *Ratibida*). Only *Bromus* and *Solanum* were present over the full range in subambient CO_2 concentrations in May 1999. For each of these species, A/g increased by 70% with the 71% increase in CO_2 from 210 to 360 μmol mol^{-1}.

Table 2 Mean values of discrimination (Δ as calculated from Eqn. 2) and parameters from linear regression analysis of relationships between Δ and CO_2 concentration (μmol mol^{-1}) for C3 species grown across a subambient gradient in CO_2 concentration. Data are from upper canopy leaves of annual grasses (*Bromus japonicus, Hordeum pusillum, Limnodea arkansana*), a perennial grass (*Nassella leucotricha*) and perennial forbs (*Ambrosia confertiflora, Medicago lupulina, Ratibida columnaris, Solanum dimidiatum* and *Solidago canadensis*) collected during 1998–2000. There was a quadratic relationship between Δ and CO_2 for *Bromus* in May 1998 ($P < 0.0001$, $r^2 = 0.72$)

Date/species	Mean Δ	*n*	*P*-value	Slope (r^2)
May 1998				
Ambrosia	20.29	5	0.004	0.018 (0.95)
Bromus	21.20	17	0.40	–
Medicago	20.36	17	0.03	−0.007 (0.28)
Nassella	21.10	10	0.14	–
Ratibida	20.85	7	0.47	–
Solanum	19.45	19	0.40	–
September 1998				
Ambrosia	19.92	4	0.06	0.007 (0.89)
Ratibida	19.16	5	0.37	–
Solanum	18.83	19	0.83	–
May 1999				
Bromus	20.46	15	0.88	–
Medicago	20.09	12	0.75	–
Ratibida	19.75	7	0.22	–
Solanum	18.63	15	0.93	–
Solidago	18.74	8	0.22	–
September 1999				
Solanum	18.96	15	0.56	–
Solidago	19.03	7	0.03	−0.01 (0.66)
April 2000				
Bromus	19.52	18	0.59	–
Hordeum	19.16	8	0.34	–
Limnodea	19.50	12	0.66	–
Solanum	17.79	15	0.01	−0.01 (0.38)

Contrary to our prediction, Δ was relatively insensitive to season (Table 2). Discrimination values declined significantly from spring to autumn in 1998 for the two most abundant perennial species sampled on both dates (*Solanum* and *Ratibida*, $P = 0.006$), but Δ of *Solanum* and *Solidago* did not differ significantly between the dry autumn and the wetter spring in 1999 ($P = 0.16$). Our hypothesis that the Δ would decline more at subambient CO_2 concentration than near the current CO_2 concentration when soils dried in autumn thus was not supported. Just the opposite was true for *Solidago* in September 1999. Discrimination by this perennial forb declined as CO_2 concentration rose.

Discrimination was lower, on average, in *Solanum* than in most other C3 species during the spring (Table 2).

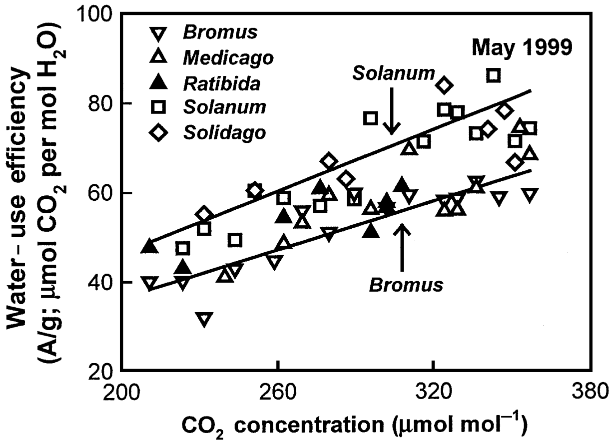

Fig. 4 Relationship between intrinsic water-use efficiency (net assimilation rate/stomatal conductance; A/g) of C3 species sampled during May 1999 and the daytime CO_2 concentration at which plants were grown. Values of A/g were derived by using c_i/c_a values calculated from stable C-isotope compositions of leaves of each species. Lines are linear regression fits of A/g on CO_2 for two of the five species only, the annual grass *Bromus japonicus* ($A/g = 0.35 + 0.18{*}CO_2$, $r^2 = 0.79$, $P = 0.0001$) and perennial forb *Solanum dimidiatum* ($A/g = 0.73 + 0.23{*}CO_2$, $r^2 = 0.80$, $P = 0.0001$). Remaining species are perennial forbs, full names of which are listed in the legend of Fig. 3.

Across subambient CO_2 concentrations, Δ was significantly lower in *Solanum* than in the other C3 species sampled in May 1998 and in April 2000 (linear contrasts, $P < 0.0001$ on each date). The Δ was significantly smaller in *Solanum* and *Solidago* than in the other C3 species in May 1999 (linear contrasts, $P < 0.0001$), when Δ was an average of 1.83‰ less in *Solanum* than in *Bromus*. Because Δ of neither *Solanum* nor *Bromus* changed significantly with CO_2 concentration, relative differences in A/g between these species remained unchanged across CO_2 concentrations. The absolute advantage in A/g of *Solanum* over *Bromus*, however, increased by about 69% from 210 to 360 μmol mol^{-1} CO_2 (Fig. 4).

Carbon dioxide effects on soil water

We calculated net depletion of water over various soil depths in each of the 5 m compartments of CO_2 chambers by subtracting the average minimum water content of each soil profile from the average maximum water content at the beginning of the season when CO_2 control was initiated (each mean is the average of three [1997] or five measurements on consecutive weeks [1998, 1999]). Relationships between water depletion and CO_2 contained considerable scatter, but significant relationships usually were best characterized as linear (Table 3). In 1998, for example, depletion of water to both 0.9 (Fig. 5) and 1.35 m depths (Table 3) declined linearly with increasing CO_2 concentration. The effects of CO_2 on soil

Table 3 Results of regression analyses for significant relationships between maximum depletion of soil water by grassland and CO_2 over subambient to superambient concentrations. Net depletion of water from soil in each 5 m long compartment of CO_2 chambers was calculated each year by subtracting the average minimum water content of soil during drought from the average maximum water content during the period of CO_2 regulation. Relationships between water depletion (y; cm H_2O) and CO_2 concentration (x; μmol mol^{-1}) were best fit by linear ($y = ax + b$) or hyperbolic [$y = ax/(b + x)$] functions

Year/depth	Model type	Slope or a-value	Intercept or b-value	r^2	*P*-value
1997					
0–1.35 m	Hyperbolic	11.55	−81.64	0.44	0.002
1998					
0–0.45 m	Linear	−0.002	5.93	0.19	0.06
0.45–0.9 m	Linear	−0.005	8.77	0.32	0.01
0–0.9 m	Linear	−0.008	15.00	0.49	0.0005
0–1.35 m	Linear	−0.017	22.90	0.63	< 0.0001
1999					
0–1.35 m	Linear	−0.007	16.91	0.27	0.02

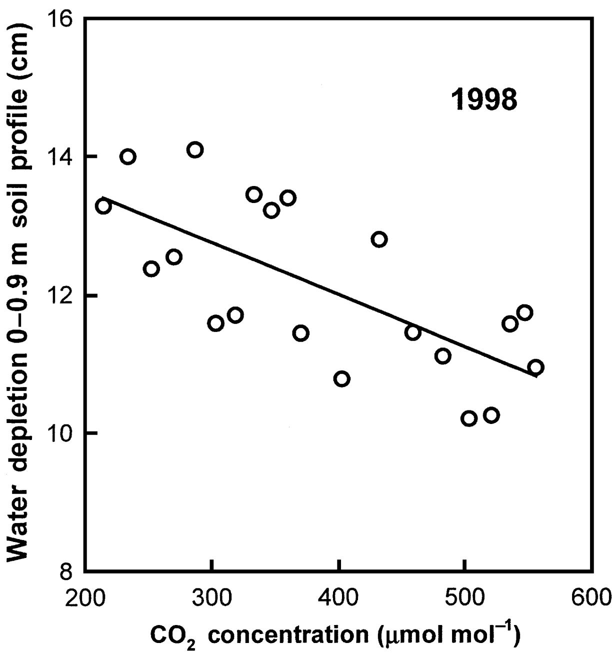

Fig. 5 Maximum depletion of soil water to 0.9 m depth during 1998 in a C3/C4 grassland exposed to subambient to superambient CO_2 concentrations. Water depletion in each of the 5 m^2 compartments of grassland in CO_2 chambers was calculated by subtracting the average minimum water content of each soil profile from the average maximum water content following initiation of CO_2 control each year (each mean is the average of five measurements on consecutive weeks). The line was fit by linear regression ($r^2 = 0.49$, $P = 0.0005$).

water in 1998 were best expressed over the 0.45–0.90 m depth. Depletion of water from shallower depths decreased marginally ($P = 0.06$) with increasing CO_2 concentration. In 1997 and 1999, water depleted to 1.35 m depth during late-season droughts correlated significantly with CO_2 treatment. In 1997, the relationship between water depletion and CO_2 was curvilinear, with greater water use over subambient concentrations than over superambient concentrations. Water depletion to 1.35 m depth decreased linearly over subambient to superambient CO_2 concentrations in 1999.

Discussion

Plant and soil water

We predicted that CO_2 enrichment would lessen soil-water depletion and increase leaf xylem potentials during periodic droughts in a C3/C4 grassland and that these responses to CO_2 would be greater over subambient concentrations than over superambient concentrations. Because leaf A/g of C3 species typically increases as soils dry (Smedley *et al*., 1991), we hypothesized that improvements in plant-water relations (less negative xylem potentials) at higher CO_2 concentrations would reduce a CO_2-mediated increase in A/g. As expected, water depletion during droughts declined and Ψ_m of dominant species became less negative as CO_2 concentration increased. Xylem potentials of the dominant grass improved slightly more over subambient concentrations than over superambient concentrations near the end of seasonal droughts in each year, but significant changes in water parameters (over subambient to superambient concentrations) and in leaf A/g (over subambient to ambient concentrations) in this grassland usually were not greater over low CO_2 concentrations than over higher CO_2 concentrations.

Our prediction that soil- and plant-water relations would become less sensitive to CO_2 as concentration increased was based on two assumptions. We assumed that transpiration tracks changes in stomatal conductance and that conductance declines more per unit increase in CO_2 over subambient concentrations than over superambient concentrations. The latter trend of greater change in conductance over low CO_2 levels than high CO_2 levels is typical of herbaceous plants grown in controlled environments (Morison, 1987). Anderson *et al*. (2001) observed a similar response of conductance among dominant species in the grassland studied here. Declines in conductance of *Bothriochloa* and *Solanum* were much larger over subambient CO_2 concentrations than over superambient CO_2 concentrations during two of the three years. During each of these years, conductance of the two dominant species declined by more than 60% as CO_2 rose from 200 to 360 μmol mol^{-1}. By contrast, conductance was a linear function of CO_2 concentration for the annual grass *Bromus* and during one of the three years for *Bothriochloa*.

Our assumption that responses of transpiration to CO_2 concentration mirror those of stomatal conductance apparently was not supported, however. Unlike changes in conductance (Anderson *et al*., 2001), measured shifts in soil-water content and in xylem potentials along the CO_2 gradient usually were small. Mid-day xylem potentials, for example, became less negative at higher CO_2 concentrations—especially for the dominant C4 grass—but the improvement was minor and inconsistent. It is well appreciated that stomatal control of transpiration and evapotranspiration (ET) is never complete (Field *et al*., 1995). Processes that operate at scales ranging from the leaf to the region tend to suppress the sensitivity of ET to stomatal closure. In this as in the other CO_2 experiments, stomatal effects on ET may have been partially offset by an increase in leaf area or in soil evaporation, by an increase in leaf temperature and in the vapour pressure difference between leaves and air and by a change in the composition or relative abundances of plant species. Expression of these feedbacks on ET, no doubt, was influenced by the climatological effects of our chambers. Chambers typically reduce wind speeds and turbulence (Owensby *et al*., 1993) and thereby increase aerodynamic resistance to convective heat transfer (Ham *et al*., 1995). Owensby *et al*. (1993) reported that open-topped chambers alone accounted for a 14% reduction in transpiration in their CO_2 study in tallgrass prairie, despite the presence of higher air and dew-point temperatures and decrease in net radiation inside the chambers.

Among feedbacks that reduced benefits of CO_2 enrichment during drought was an increase in growth at higher CO_2 concentrations. Daily means of net CO_2 uptake for this grassland were markedly higher over superambient CO_2 concentrations than over subambient CO_2 concentrations during the 1998 and 1999 growing seasons, and were linearly correlated with end-of-season biomass in each year (Mielnick *et al*., 2001). Water savings resulting from partial stomatal closure apparently were at least partly offset at higher CO_2 concentrations by this increase in biomass that presumably also increased leaf area.

Relatively small responses of soil and plant water to CO_2 probably also resulted from our inability to completely control the water balance of this grassland. There was evidence, for instance, that water moved from soil outside of chambers into soil immediately beneath CO_2 chambers, despite the presence of a physical barrier to water movement to almost 1 m depth. Rains totalling 4 cm fell on days 130 and 131 in 1999. Between days 126 and 133, water content of soil beneath chambers increased by an average of 0.84 cm to 1.35 m depth despite the absence of irrigation. The net increase in water content of the 0–0.9 m soil profile that was delimited from surrounding soil was smaller, averaging 0.38 cm or 1.3% of average water content to 0.9 m depth. There was no relationship between CO_2 treatment and the increase in water content of chambered soils following rainfall (not shown), but availability of this additional water undoubtedly reduced the severity of water stress experienced by plants along the CO_2 gradient.

Though the effects of drought were relatively minor in this study, CO_2 enrichment reduced soil-water depletion during the mid-season drought in 1998 and during the late-season droughts in 1997 and 1999 and improved Ψ_m of dominant plants during each of the three years. Our results thus provide the first field evidence for a continuous change in water dynamics over subambient to superambient CO_2 concentrations.

It, perhaps, is noteworthy that CO_2 effects on the rate of soil-water decline were particularly well expressed during the mid-season drought in 1998. In order to reduce the rate of soil-water depletion, CO_2 enrichment must reduce transpiration. The absolute magnitude of any decline in transpiration at high CO_2 is a function both of the transpiration rate itself and of the relative decrease in water loss at higher CO_2 concentrations. Net CO_2 uptake by the C3/C4 grassland studied peaked in late-May of 1998 (~day 150), about mid-way through the drought period (Mielnick *et al*., 2001), suggesting that transpiration rates also were high during the initial stages of soil-water depletion. As indicated by seasonal changes in slopes of relationships between air $\delta^{13}C$ values and CO_2 over subambient concentrations, C3 contribution to net photosynthesis (and, presumably to transpiration) in this grassland also was greatest during early to mid-season. Stomatal conductance of both C3 and C4 species declined at higher CO_2, but the absolute magnitude of the reduction was greater by a factor of 3–4 among the C3 species than for the dominant C4 grass (Anderson *et al*., 2001). The combination of high transpiration rates and large declines in stomatal conductance of dominant plants at higher CO_2 concentrations may have contributed to the reduction in water use at high CO_2 during 1998.

Because CO_2 treatments were not replicated in this experiment, measured responses may have been influenced by unquantified factors that varied with CO_2 concentration. Three lines of evidence indicate that the influence of variables other than CO_2 on measured responses was minimal. First, soil-water content did not differ along chambers during the year preceding fumigation. Second, the environmental factors remained similar across CO_2 gradients (Johnson *et al*., 2000). Third, responses to CO_2 generally were continuous across the full gradient. Near-ambient CO_2 concentrations were maintained at the northern (air exit) extreme of one chamber and at the southern (air entrance) extreme of the second chamber. That responses to CO_2 were continuous, therefore, is evidence that neither landscape position nor position within chambers appreciably influenced the results.

Intrinsic water use efficiency

Comparisons of A/g for leaves sampled at different times during the growing season do not provide a quantitative measure of differences in plant water-use efficiency, because water-use efficiency is influenced by evaporative demand of the atmosphere and by C losses to respiration. Temperature and evaporative demand increased from spring to autumn in this experiment (Johnson *et al*., 2000), suggesting that for a given Δ, water-use efficiency was lower in autumn than in spring. Across subambient CO_2 concentrations, Δ-values for *Solanum* and *Solidago* did not differ between spring and autumn in 1999, implying that, contrary to prediction, water-use efficiency of these perennial forbs declined as the season advanced.

The results of this experiment provide field confirmation that A/g of herbaceous species responds linearly to CO_2 over subambient concentrations, as was demonstrated in a controlled environment (Polley *et al*., 1993). Leaf-gas exchange measurements on grassland dominants indicated that the increase in A/g extended over the full subambient to superambient gradient in CO_2 concentration (Anderson *et al*., 2001). Both lower g and higher A contributed to the increase in A/g of C3 and C4 species. Because Δ and c_i/c_a were conservative across CO_2 concentrations, the increase in A/g was nearly proportional to that in CO_2 concentration. Feedbacks may reduce expression of this increase in leaf A/g at regional scales (Field *et al*., 1995), but our results indicate that water-use efficiency of herbaceous species already may be substantially greater than it was during most of the last 420 000 years when CO_2 concentrations were low (Petit *et al*., 1999). The continuous responses of leaf A/g, Ψ_m and soil-water depletion to CO_2 in this grassland also indicate that water-limited ecosystems may remain sensitive to rising CO_2 concentration.

Mean values of Δ invariably were smaller in the perennial forb *Solanum* than in the annual grasses, indicating that A/g was greater in *Solanum* than in annuals. This pattern is consistent with the negative correlation between Δ and life span reported in other studies (Ehleringer & Cooper, 1988; Schuster *et al*., 1992), but the pattern was not evident in A/*g*-values calculated from leaf-gas exchange measurements (Anderson *et al*., 2001). The A/g derived from gas exchange was greater in the annual grass *Bromus* than in *Solanum* during the first year and did not differ greatly between species during a second year. Why gas exchange and leaf $\delta^{13}C$ measurements yielded different values of A/g is not clear, but disparities may reflect differences in the temporal scale of the two methods. Gas exchange is measured almost instantaneously. Carbon isotope values integrate over the period of leaf C fixation. Nevertheless, if Δ remains nearly constant across CO_2 concentrations as demonstrated here, proportional differences in A/g among species will not change as CO_2 rises, but absolute differences in A/g will be exaggerated at higher CO_2 concentrations. Production in water-limited systems may, therefore, become even more sensitive to shifts in species relative abundances as CO_2 rises.

Acknowledgements

Ron Whitis maintained CO_2 chambers and measured soil-water content. Katherine Jones and Chris Kolodziejczyk measured xylem potentials and prepared samples for isotope analyses. William Dugas, Richard Gill, Robert Jackson, Hafiz Maherali, Patricia Mielnick and Chantal Reid provided helpful reviews of the manuscript.

References

Anderson LJ, Maherali H, Johnson HB *et al.* (2001) Gas exchange and photosynthetic acclimation over subambient to elevated CO_2 in a C3–C4 grassland. *Global Change Biology*, **7**, 693–707.

Condon AG, Richards RA, Farquhar GD (1992) The effect of variation in soil water availability, vapour pressure deficit and nitrogen nutrition on carbon isotope discrimination in wheat. *Australian Journal of Agricultural Research*, **43**, 935–947.

Ehleringer JR, Cooper TA (1988) Correlations between carbon isotope ratio and microhabitat in desert plants. *Oecologia*, **76**, 562–566.

Farquhar GD, O'Leary MH, Berry JA (1982) On the relationship between carbon isotope discrimination and intercellular carbon dioxide concentration in leaves. *Australian Journal of Plant Physiology*, **9**, 121–137.

Field CB, Jackson RB, Mooney HA (1995) Stomatal responses to increased CO_2: implications from the plant to the global scale. *Plant, Cell and Environment*, **18**, 1214–1225.

Field CB, Lund CP, Chiariello NR *et al.* (1997) CO_2 effects on the water budget of grassland microcosm communities. *Global Change Biology*, **3**, 197–206.

Fredeen AL, Randerson JT, Holbrook NM *et al.* (1997) Elevated atmospheric CO_2 increases water availability in a water-limited grassland ecosystem. *Journal of the American Water Resources Association*, **33**, 1033–1039.

Ham JM, Owensby CE, Coyne PI *et al.* (1995) Fluxes of CO_2 and water vapor from a prairie ecosystem exposed to ambient and elevated atmospheric CO_2. *Agricultural and Forest Meteorology*, **77**, 73–93.

Hamerlynck EP, McAllister CA, Knapp AK *et al.* (1997) Photosynthetic gas exchange and water relation responses of three tallgrass prairie species to elevated carbon dioxide and moderate drought. *International Journal of Plant Sciences*, **158**, 608–616.

Jackson RB, Sala OE, Field CB *et al.* (1994) CO_2 alters water use, carbon gain, and yield for the dominant species in a natural grassland. *Oecologia*, **98**, 257–262.

Johnson HB, Polley HW, Whitis RP (2000) Elongated chambers for field studies across atmospheric CO_2 gradients. *Functional Ecology*, **14**, 388–396.

Knapp AK, Hamerlynck EP, Ham JM *et al.* (1996) Responses of stomatal conductance to elevated CO_2 in 12 grassland species that differ in growth form. *Vegetatio*, **125**, 31–41.

Maherali H, Reid CD, Polley HW *et al.* (2002) Stomatal acclimation over a subambient to elevated CO_2 gradient in a C3/C4 grassland. *Plant, Cell and Environment*, **25**, 557–566.

Meinzer FC, Goldstein G, Grantz DA (1990) Carbon isotope discrimination in coffee genotypes grown under limited water supply. *Plant Physiology*, **92**, 130–135.

Mielnick PC, Dugas WA, Johnson HB *et al.* (2001) Net grassland carbon flux over a subambient to superambient CO_2 gradient. *Global Change Biology*, **7**, 747–754.

Morgan JA, LeCain DR, Mosier AR *et al.* (2001) Elevated CO_2 enhances water relations and productivity and affects gas exchange in C3 and C4 grasses of the Colorado shortgrass steppe. *Global Change Biology*, **7**, 451–466.

Morison JIL (1987) Intercellular CO_2 concentration and stomatal response to CO_2. In: *Stomatal Function* (eds Zeiger E, Farquhar GD, Cowan I), pp. 229–251. Stanford University Press, Stanford, CA, USA.

Niklaus PA, Spinnler D, Körner C (1998) Soil moisture dynamics of calcareous grassland under elevated CO_2. *Oecologia*, **117**, 201–208.

Owensby CE, Coyne PI, Ham JM *et al.* (1993) Biomass production in a tallgrass prairie ecosystem exposed to ambient and elevated CO_2. *Ecological Applications*, **3**, 644–653.

Owensby CE, Ham JM, Knapp AK *et al.* (1999) Biomass production and species composition change in a tallgrass prairie ecosystem after long-term exposure to elevated atmospheric CO_2. *Global Change Biology*, **5**, 497–506.

Owensby CE, Ham JM, Knapp AK *et al.* (1997) Water vapour fluxes and their impact under elevated CO_2 in a C4-tallgrass prairie. *Global Change Biology*, **3**, 189–195.

Pataki DE, Huxman TE, Jordan DN *et al.* (2000) Water use of two Mojave Desert shrubs under elevated CO_2. *Global Change Biology*, **6**, 889–897.

Petit JR, Jouzel J, Raynaud D *et al.* (1999) Climate and atmospheric history of the past 420 000 years from the Vostok ice core, Antarctica. *Nature*, **399**, 429–436.

Polley HW, Johnson HB, Marino BD *et al.* (1993) Increase in C3 plant water-use efficiency and biomass over Glacial to present CO_2 concentrations. *Nature*, **361**, 61–64.

Polley HW, Johnson HB, Mayeux HS *et al.* (1996) Impacts of rising CO_2 concentration on water use efficiency of woody grassland invaders. In: *Proceedings: Shrubland Ecosystem Dynamics in a Changing Environment* (eds Barrow JR, McArthur ED, Sosebee RE, Tausch RJ), pp. 189–194. Intermountain Research Station, USDA Forest Service, Ogden, Utah.

Polley HW, Mayeux HS, Johnson HB *et al.* (1997) Viewpoint: Atmospheric CO_2, soil water, and shrub–grass ratios on rangelands. *Journal of Range Management*, **50**, 278–284.

Sage RF (1994) Acclimation of photosynthesis to increasing atmospheric CO_2: The gas exchange perspective. *Photosynthesis Research*, **39**, 351–368.

Schapendonk AHCM, Dijkstra P, Groenwold J *et al.* (1997) Carbon balance and water use efficiency of frequently cut *Lolium perenne* L. swards at elevated carbon dioxide. *Global Change Biology*, **3**, 207–216.

Schuster WSF, Sandquist DR, Phillips SL *et al.* (1992) Comparisons of carbon isotope discrimination in populations of aridland plant species differing in lifespan. *Oecologia*, **91**, 332–337.

Smedley MP, Dawson TE, Comstock JP *et al.* (1991) Seasonal carbon isotope discrimination in a grassland community. *Oecologia*, **85**, 314–320.

Tissue DT, Griffin KL, Thomas RB *et al.* (1995) Effects of low and elevated CO_2 on C3 and C4 annuals. II. Photosynthesis and leaf biochemistry. *Oecologia*, **101**, 21–28.