

# Gas exchange and photosynthetic acclimation over subambient to elevated CO<sub>2</sub> in a C<sub>3</sub>–C<sub>4</sub> grassland

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## Abstract

Atmospheric CO<sub>2</sub> (C<sub>a</sub>) has risen dramatically since preglacial times and is projected to double in the next century. As part of a 4-year study, we examined leaf gas exchange and photosynthetic acclimation in C<sub>3</sub> and C<sub>4</sub> plants using unique chambers that maintained a continuous C<sub>a</sub> gradient from 200 to 550 μmol mol<sup>-1</sup> in a natural grassland. Our goals were to characterize linear, nonlinear and threshold responses to increasing C<sub>a</sub> from past to future C<sub>a</sub> levels. Photosynthesis (A), stomatal conductance (g<sub>s</sub>), leaf water-use efficiency (A/g<sub>s</sub>) and leaf N content were measured in three common species: *Bothriochloa ischaemum*, a C<sub>4</sub> perennial grass, *Bromus japonicus*, a C<sub>3</sub> annual grass, and *Solanum dimidiatum*, a C<sub>3</sub> perennial forb. Assimilation responses to internal CO<sub>2</sub> concentrations (A/C<sub>i</sub> curves) and photosynthetically active radiation (A/PAR curves) were also assessed, and acclimation parameters estimated from these data. Photosynthesis increased linearly with C<sub>a</sub> in all species (P < 0.05). *S. dimidiatum* and *B. ischaemum* had greater carboxylation rates for Rubisco and PEP carboxylase, respectively, at subambient than superambient C<sub>a</sub> (P < 0.05). To our knowledge, this is the first published evidence of A up-regulation at subambient C<sub>a</sub> in the field. No species showed down-regulation at superambient C<sub>a</sub>. Stomatal conductance generally showed curvilinear decreases with C<sub>a</sub> in the perennial species (P < 0.05), with steeper declines over subambient C<sub>a</sub> than superambient, suggesting that plant water relations have already changed significantly with past C<sub>a</sub> increases. Resource-use efficiency (A/g<sub>s</sub> and A/leaf N) in all species increased linearly with C<sub>a</sub>. As both C<sub>3</sub> and C<sub>4</sub> plants had significant responses in A, g<sub>s</sub>, A/g<sub>s</sub> and A/leaf N to C<sub>a</sub> enrichment, future C<sub>a</sub> increases in this grassland may not favour C<sub>3</sub> species as much as originally thought. Non-linear responses and acclimation to low C<sub>a</sub> should be incorporated into mechanistic models to better predict the effects of past and present rising C<sub>a</sub> on grassland ecosystems.

**Keywords:** subambient CO<sub>2</sub>, elevated CO<sub>2</sub>, photosynthetic acclimation, up-regulation, photosynthesis, stomatal conductance, resource-use efficiency, grassland

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## Introduction

Considerable research has been devoted to understanding physiological responses of plants to future atmos-

pheric CO<sub>2</sub> (C<sub>a</sub>) increases and the resulting consequences for natural and agricultural ecosystems (reviewed in Drake *et al.* 1997; Curtis & Wang 1998; Saxe *et al.* 1998; Hsiao & Jackson 1999; Wand *et al.* 1999; Ward & Strain 1999). Much of this work has compared plants grown at ambient C<sub>a</sub> (360 μmol mol<sup>-1</sup>) with those grown at twice ambient concentrations, and has shown that increased C<sub>a</sub> enhances photosynthesis (A) and growth, decreases

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stomatal conductance ( $g_s$ ), and increases leaf water-use efficiency ( $A/g_s$ ) for a variety of species and ecosystems (e.g. Owensby *et al.* 1993; Knapp *et al.* 1993; Jackson *et al.* 1994, 1995). However, nonlinear or threshold responses are difficult or impossible to assess in these experiments because of the limited number of  $C_a$  concentrations examined. Recent modelling work (Ackerly & Bazzaz 1995; Luo & Reynolds 1999) and a few empirical studies at three or four  $C_a$  concentrations (e.g. Hunt *et al.* 1991, 1993) suggest that single species, community and ecosystem responses to  $C_a$  are frequently nonlinear.  $C_a$  is increasing gradually, and we cannot necessarily interpolate responses to intermediate  $C_a$  concentrations based on data at twice ambient levels. Additional experiments on plant responses over the entire  $C_a$  gradient are needed to refine our predictions of ecosystem responses to future  $C_a$  increases (Ackerly & Bazzaz 1995; Luo & Reynolds 1999).

Ambient-superambient comparisons also do not take into account past increases in  $C_a$  that have already shaped vegetation. Ice core data show that  $C_a$  fluctuated from 180 to 300  $\mu\text{mol mol}^{-1}$  during the last 250 000 years (reviewed in Sage & Cowling 1999), and was sometimes below 200  $\mu\text{mol mol}^{-1}$  for 10 000-year periods (Barnola *et al.* 1987; Jouzel *et al.* 1993). Past  $C_a$  increases have been associated with changes in the global distributions of  $C_3$  and  $C_4$  plants (e.g. Johnson *et al.* 1993; Ehleringer *et al.* 1997), increases in ecosystem productivity (e.g. Phillips & Gentry 1994), and the advent of agriculture (Sage 1995), but we know relatively little about the physiological responses of plants to subambient  $C_a$ , and how they compare with responses to superambient  $C_a$ . Examining plant sensitivity to a range of  $C_a$  concentrations may give insight into the physiological capacity of plants to respond to  $C_a$  increases, both past and future (Sage & Cowling 1999; Ward *et al.* 2000).

Our study examined leaf gas exchange in field-grown  $C_3$  and  $C_4$  plants using unique experimental chambers that maintained a continuous gradient of  $C_a$  from 200 to 550  $\mu\text{mol mol}^{-1}$  in a natural grassland community. While there has been some work with subambient  $C_a$  in crops (e.g. Baker *et al.* 1990a,b; Campbell *et al.* 1990; Rowland-Bamford *et al.* 1990; Allen *et al.* 1991; Mayeux *et al.* 1997) and wild  $C_3$  plants in growth chambers (e.g. Overdieck 1989; Polley *et al.* 1992a,b; 1995), no studies at subambient  $C_a$  have examined intact plant communities. Furthermore, few have compared physiological responses in  $C_3$  and  $C_4$  species.  $C_4$  plants are predicted to have a competitive advantage over  $C_3$  plants at past low  $C_a$  levels, while  $C_a$  mediated increases in  $C_3$  quantum yield are expected to favour  $C_3$  over  $C_4$  plants in the future (Ehleringer *et al.* 1997). The few studies at subambient  $C_a$  using both  $C_3$  and  $C_4$  plants found reduced growth,  $A$  and  $A/g_s$ , and increased  $g_s$  for  $C_3$

species (Polley *et al.* 1992b, 1993; Dippery *et al.* 1995; Tissue *et al.* 1995). In contrast, the  $C_4$  plants had minimal growth responses to low  $C_a$ , but some showed increased  $g_s$  and reduced  $A/g_s$ . The growth results are consistent with the expectation that  $A$  is less sensitive to  $C_a$  in  $C_4$  than  $C_3$  plants (but see Wand *et al.* 1999 and Ghannoum *et al.* 2000).

Although  $A$  is usually stimulated by rising  $C_a$ , there is wide variation in species' responses. One source of this variation is acclimation of  $A$  to  $C_a$ . We define acclimation as a physiological adjustment to a given  $C_a$ , particularly up- or down-regulation of  $A$  through adjustments in photosynthetic biochemistry (see Sage 1994). Assuming the balance between carbohydrate sources and sinks regulates acclimation, we might expect superambient  $C_a$  to cause down-regulation of  $A$  by increasing the source/sink ratio, and subambient  $C_a$  to cause up-regulation by decreasing the source/sink ratio (Sage & Cowling 1999). Down-regulation of  $A$  at superambient  $C_a$  is not common in the field, but is often seen in greenhouse studies (Sage 1994), possibly because sink demands are reduced by root restriction in pots (Thomas & Strain 1991). Up-regulation at subambient  $C_a$  has not been observed (Thomas & Strain 1991; Sage & Reid 1992; Tissue *et al.* 1995; Cowling & Sage 1998; but see Gesch *et al.* 2000), but has also not been studied in the field where sink demands may be greater.

In this study, we report physiological responses of grassland species to a continuous gradient of past and future  $C_a$  concentrations. We were interested in photosynthetic acclimation and comparing gas exchange responses over sub- and superambient  $C_a$ . We focused on three abundant species with different growth forms and physiological pathways: *Bothriochloa ischaemum* (L.) Keng, a  $C_4$  perennial grass, *Bromus japonicus* L., a  $C_3$  annual grass, and *Solanum dimidiatum* Raf., a  $C_3$  perennial forb. We measured gas exchange parameters ( $A$ ,  $g_s$  and leaf-level  $A/g_s$ ) in plants along the  $C_a$  gradient over two growing seasons. We also measured assimilation responses to internal  $\text{CO}_2$  concentrations ( $A/C_i$  curves) and photosynthetically active radiation ( $A/\text{PAR}$  curves) for each species. Acclimation and quantum yield parameters were estimated from these data to explore physiological mechanisms underlying gas exchange patterns.

## Materials and methods

### Study site

The experiment took place in a grassland at the USDA/ARS Grassland, Soil and Water Research Laboratory in Temple, Texas, USA (31°05' N, 97°20' W). The site has been managed as grassland for at least 50 years, and was last grazed by cattle in 1992. The vegetation was a diverse

**Table 1** Measurement dates and LI-6400 leaf chamber conditions for three species along the C<sub>a</sub> gradient. It was not logistically possible to measure plants in all sections of the experimental chambers on one day. Therefore, on each day plants at different points along the C<sub>a</sub> gradient were randomly selected for gas exchange measurements, assuring that a wide range of the gradient was represented. Data from different days of a given measurement session (e.g. 8–13 April 1999) were pooled to characterize the gas exchange responses of a species at that point in the season. 'AC' and 'AP' indicate dates when A/C<sub>i</sub> and A/PAR curves, respectively, were measured. LAVPD = leaf-to-air vapour pressure deficit. The leaf temperatures and LAVPDs maintained in the LI-6400 chamber reflect the different times of year and environmental conditions during which species were active.

Species and LI-6400 chamber conditions	Spring measurement dates	Summer measurement dates
<i>Bromus japonicus</i> (C <sub>3</sub> annual grass) Saturating irradiance (μmol m <sup>-2</sup> s <sup>-1</sup> ): 1200 Leaf temperature (°C): 20–23 LAVPD (kPa): 1.1–1.5 in 1999 0.9–1.1 in 2000	8–13 April 1999, AC 20–25 April 2000, AP	No plants present
<i>Solanum dimidiatum</i> (C <sub>3</sub> perennial forb) Saturating irradiance (μmol m <sup>-2</sup> s <sup>-1</sup> ): 1600 Leaf temperature (°C): 27–30 LAVPD (kPa): 0.9–1.1		24–27 June 1999, AP 8–14 July 1999, AC 11–12 June 2000
<i>Bothriochloa ischaemum</i> (C <sub>4</sub> perennial grass) Saturating irradiance (μmol m <sup>-2</sup> s <sup>-1</sup> ): 2000 Leaf temperature (°C): 29–32 LAVPD (kPa): 1.4–1.6		22–27 August 1999, AP, AC 21–30 August 2000

mixture of native and introduced grasses and forbs. Dominant plants included *B. ischaemum*, *S. dimidiatum*, and the C<sub>3</sub> perennial forb *Ratibida columnaris* (Sims) D. Don. Mean annual precipitation at the site is 877 mm (1913–99), and the mean maximum and minimum annual temperatures are 25.9 °C and 13.2 °C, respectively (1914–95, USDA/ARS Grassland, Soil and Water Research Laboratory weather station records). The soil is a mollisol in the Austin series (classified as a fine-silty, carbonatic, thermic, Udorthentic Haplustoll) with 35–55% clay in the top 40 cm. Inclusions of the Houston series (a vertisol defined as a fine, smectitic, thermic, Udic Haplustert) are common.

#### Experimental field chambers

The experimental chambers are described in detail in Johnson *et al.* (2000) and provide a continuous gradient of C<sub>a</sub> from 200 to 360 μmol mol<sup>-1</sup> in one chamber (subambient chamber) and from 360 to 550 μmol mol<sup>-1</sup> in the other (superambient chamber). The chambers were built over parallel, adjacent plots of grassland each 60-m long, 1-m wide, and 1.5-m apart. Each chamber was divided into 10 continuous 5-m sections with chiller and condenser units connecting consecutive sections. Chambers were 1-m tall and constructed of polyethylene film, which transmitted 85–95% of incident photosynthetic photon flux density (PPFD). A large fan at the end of each chamber blew in ambient air. In the super-

ambient chamber, incoming air was enriched with CO<sub>2</sub> to give a concentration of 550 μmol mol<sup>-1</sup>. As the air moved down each chamber, plant A reduced C<sub>a</sub> to 360 μmol mol<sup>-1</sup> at the end of the superambient chamber and to 200 μmol mol<sup>-1</sup> at the end of the subambient chamber. As A varied with light, water and plant biomass, air flow rates in the chambers were automatically adjusted by increasing or decreasing fan speeds to accommodate A changes. At night, C<sub>a</sub> gradients were maintained at 150 μmol mol<sup>-1</sup> above daytime levels by reversing air flow and using respiratory CO<sub>2</sub> releases to create the gradient.

A rubber-coated fabric barrier extended 0.9-m deep into the soil along the chamber sides. Irrigation was applied equally to each 5-m section to match rainfall outside through July 1999. After this, irrigation was applied such that soil water content in sections at ambient C<sub>a</sub> matched that of adjacent grassland outside as measured by neutron attenuation. The total water applied to the chambers was 349 and 381 mm in the very dry years of 1999 and 2000 (up until 13 September), respectively. Atmospheric humidity (RH) and temperature (T) along the C<sub>a</sub> gradient were re-set with chilled-water condensers placed in the 1-m area between 5-m chamber sections. Direct, instantaneous measurements of T and RH during leaf gas exchange measurements in 1998 did not show any consistent differences in these variables along the gradient (L. J. Anderson, unpublished data). Averaged across sections, daytime

air temperatures were generally 2–4 °C below ambient and were similar in the two chambers, while mean monthly vapour pressure deficits were slightly higher in the subambient chamber than in the superambient during summer (Johnson *et al.* 2000).

The chambers were activated in May (1997); and have been operating each growing season (mid-February to mid-November) since then.  $C_a$  gradients have been maintained for > 90% of growing season days, even during severe droughts (Johnson *et al.* 2000). The plastic cover was removed from the chambers for a few months each winter, when vegetation was dormant.

#### *Steady state leaf gas exchange*

Leaf gas-exchange was measured for *B. japonicus*, *S. dimidiatum* and *B. ischaemum* along the  $C_a$  gradient during the 1999 and 2000 growing seasons (Table 1). For each species, measurements were done at a minimum of six treatment  $C_a$  concentrations (six chamber sections) on three to four plants per section. Measurements were made on the youngest, fully expanded leaves with an open gas-exchange system (LI-6400, Li-Cor Inc., Lincoln, NE, USA) between 0900 and 1500 h central standard time. Incident irradiance during measurements was maintained at saturating levels by red-blue light-emitting diodes, and a Peltier cooling module controlled leaf temperatures (Table 1). To calculate  $g_s$  and intercellular  $CO_2$  concentration ( $C_i$ ) for the grasses, we used a boundary layer conductance of  $2.84 \text{ mol m}^{-2} \text{ s}^{-1}$  for amphistomatous leaves. For *S. dimidiatum* we used a value of  $1.42 \text{ mol m}^{-2} \text{ s}^{-1}$  for hypostomatous leaves. Leaf areas were measured with the LI-3000A portable leaf area meter (Li-Cor, Inc.) or from leaf dimensions, depending on sample morphology.

#### *Apparent quantum yield and photosynthetic acclimation*

Photosynthetic responses to incident irradiance ( $A/PAR$  curves) for *S. dimidiatum*, *B. ischaemum* and *B. japonicus* were measured in June 1999, August 1999 and April 2000, respectively, using the same leaves as in the survey measurements above (Table 1). To examine the effect of  $C_a$  on apparent quantum yield, we made multiple measurements at irradiances below  $120 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$ , where the slope of the curve is approximately linear. The apparent quantum yield was calculated as the slope of the  $A/PAR$  curve between 10 and  $100 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$ . All  $A/PAR$  curves were measured at the treatment  $C_a$  concentration. During measurements, leaf temperatures and LAVPD were maintained as described in Table 1.

To examine photosynthetic acclimation to the  $C_a$  treatments, we measured the response of net  $A$  to calculated  $C_i$ .  $A/C_i$  curves were measured at saturating irradiances on *B. japonicus* in April, *S. dimidiatum* in July, and *B. ischaemum* in August 1999 using the same leaves as in the survey measurements (Table 1,  $C_a$  values for  $A/C_i$  curves ranged from 215 to  $546 \text{ } \mu\text{mol mol}^{-1}$ ). For the  $C_3$  species,  $A/C_i$  curves were analysed with a mechanistic two-factor model derived by Farquhar *et al.* (1980) and modified by Harley *et al.* (1992). The light saturated rate of carboxylation ( $V_{cmax}$ ) by ribulose-1,5-bisphosphate (RuBP) carboxylase (Rubisco) and maximum rate of RuBP regeneration as a function of electron transport capacity ( $J_{max}$ ) were calculated using the kinetic assumptions of Harley *et al.* (1992) with the aid of software developed by S. P. Long (University of Illinois, unpublished manuscript). For the  $C_4$  species *B. ischaemum*, the efficiency of the PEP carboxylase  $CO_2$  pump was determined from the slope of the linear portion of the  $A/C_i$  curve. Maximum  $CO_2$ -saturated  $A$  rates and the  $C_i$  at which  $A$  reached saturation were determined for *B. ischaemum* as in Tissue *et al.* (1995).

#### *Leaf nitrogen and photosynthetic nitrogen-use efficiency*

Leaves used for gas exchange in 1999 and leaves collected along the gradient in 1998 were dried at 65 °C to constant weight, and ground to a powder using a Crescent Wig-L-Bug (Crescent Dental, Lyons, Illinois). Powder samples were assessed for percentage C and N content using a CE Instruments NC 2100 elemental analyser (ThermoQuest Italia, Milan, Italy). Photosynthetic nitrogen-use efficiency (PNUE) was calculated for the 1999 samples as  $A$  ( $\mu\text{mol } CO_2 \text{ m}^{-2} \text{ s}^{-1}$ )/leaf N content ( $\text{g m}^{-2}$ ).

#### *Data analysis*

The relationship between gas exchange variables and  $C_a$  concentration was assessed using the Regression : Curve fitting procedure in SPSS 8.0 for Windows (SPSS Inc., Chicago, IL) and the Regression Wizard in Sigma Plot 5.0 for Windows (SPSS Inc., Chicago, IL). Because ours was the first study examining physiological responses to a  $C_a$  gradient in an intact plant community, we took an exploratory approach to our data analysis and tried a diversity of models. Several different, biologically reasonable responses to the  $C_a$  gradient were observed, and these responses varied over time. Simple linear, logarithmic, power and hyperbolic functions were fitted to the data and the adjusted  $R^2$  values compared to find the model with the best fit. When models had similar explanatory power ( $R^2_s$  within 0.05), the linear model

**Table 2** Regression analysis results for relationships between C<sub>a</sub> concentration and gas exchange parameters for three species in 1999 and 2000. NS = no significant relationship with C<sub>a</sub>, linear = linear model ( $y = ax + b$ ), power = power model ( $y = ax^b$ ), log = logarithmic model ( $y = a \ln x + b$ ), hyper. 1 = hyperbolic model 1 ( $y = ax/(b + x)$ ), hyper. 2 = hyperbolic model 2 ( $y = a + (b/x^2)$ ), Intercept = intercept values for regression equations.

Variable, species and measurement date	Model type	Increase or decrease with C <sub>a</sub>	Parameter value (a)	Intercept (b)	r <sup>2</sup>	P-value
<b>Photosynthesis (A)</b>						
<i>B. japonicus</i> , Apr 1999	Linear	Increase	0.0322	5.22	0.60	< 0.0001
<i>B. japonicus</i> , Apr 2000	Linear	Increase	0.0294	6.16	0.69	< 0.0001
<i>S. dimidiatum</i> , Jun 1999	Linear	Increase	0.0261	9.97	0.69	< 0.0001
<i>S. dimidiatum</i> , Jun 2000	Linear	Increase	0.0293	9.71	0.61	< 0.0001
<i>B. ischaemum</i> , Aug 1999	Linear	Increase	0.0286	12.07	0.56	0.0003
<i>B. ischaemum</i> , Aug 2000	Linear	Increase	0.0302	4.02	0.68	< 0.0001
<b>Stomatal conductance (g<sub>s</sub>)</b>						
<i>B. japonicus</i> , Apr 1999	Linear	Decrease	-0.8772	699.30	0.36	0.0018
<i>B. japonicus</i> , Apr 2000	Linear	Decrease	-1.0112	899.96	0.73	< 0.0001
<i>S. dimidiatum</i> , Jun 1999	Hyper. 1	Decrease	414.42	-168.4	0.68	< 0.0001
<i>S. dimidiatum</i> , Jun 2000	Hyper. 2	Decrease	208.92	9.1 × 10 <sup>7</sup>	0.89	< 0.0001
<i>B. ischaemum</i> , Aug 1999	Power	Decrease	8302.9	-0.6383	0.76	< 0.0001
<i>B. ischaemum</i> , Aug 2000	Linear	Decrease	-0.1632	188.88	0.66	< 0.0001
<b>Water-use efficiency (A/g<sub>s</sub>)</b>						
<i>B. japonicus</i> , Apr 1999	Linear	Increase	0.2061	-22.32	0.70	< 0.0001
<i>B. japonicus</i> , Apr 2000	Linear	Increase	0.1348	-13.75	0.87	< 0.0001
<i>S. dimidiatum</i> , Jun 1999	Linear	Increase	0.1049	-12.23	0.78	< 0.0001
<i>S. dimidiatum</i> , Jun 2000	Linear	Increase	0.1489	-27.06	0.73	< 0.0001
<i>B. ischaemum</i> , Aug 1999	Linear	Increase	0.3707	-15.53	0.82	< 0.0001
<i>B. ischaemum</i> , Aug 2000	Linear	Increase	0.4071	-26.26	0.92	< 0.0001
<b>C<sub>i</sub>/C<sub>a</sub> Ratio (Mean ± SE, Range)</b>						
<i>B. japonicus</i> , Apr 1999 (0.74 ± 0.02, 0.61–0.85)	Linear	Decrease	-0.0004	0.89	0.45	0.0010
<i>B. japonicus</i> , Apr 2000 (0.81 ± 0.01, 0.73–0.86)	Linear	Decrease	-0.0002	0.88	0.48	0.0015
<i>S. dimidiatum</i> , Jun 1999 (0.81 ± 0.01, 0.76–0.86)	Linear	Decrease	-0.0001	0.85	0.23	0.0436
<i>S. dimidiatum</i> , Jun 2000 (0.82 ± 0.01, 0.72–0.89)	Linear	Decrease	-0.0002	0.90	0.36	0.0038
<i>B. ischaemum</i> , Aug 1999 (0.35 ± 0.02, 0.25–0.48)	Linear	Decrease	-0.0003	0.47	0.31	0.0198
<i>B. ischaemum</i> , Aug 2000 (0.42 ± 0.01, 0.32–0.55)	Linear	NS	-0.0002	0.50	0.12	0.0685

was selected. Plots of residuals were examined for normality and homoscedasticity for all linear fits, and no violations of assumptions were found. As several measurements were taken within a given section of the C<sub>a</sub> gradient, we generally had more than one  $y$ -value for a given  $x$  (where  $x = C_a$  concentration). Therefore we tested the hypothesis that the data were linear using the Lack-of-Fit option in the General Linear Model procedure in SPSS 8.0 and following the guidelines in Zar (1996) for regression with replication. For clarity,  $y$ -values in figures are presented as means with standard errors, but all analyses were done on individual variates. In cases where there was no significant relationship with C<sub>a</sub>, data are plotted with a linear model. Additional lines were

hand-fit to the quantum yield data to highlight patterns not well described by the overall models for each data set. Data for each species at each time point were analysed separately (Tables 2–4), although 1999 and 2000 data described by similar models are plotted with a single line in the figures, to emphasize the consistency of the relationship with C<sub>a</sub>.

## Results

### *Patterns of gas exchange along the CO<sub>2</sub> gradient*

Photosynthetic rates increased significantly with increasing C<sub>a</sub> for all three species in 1999 and 2000, more than 2 years after C<sub>a</sub> treatments were initiated. All responses

were linear across sub- and superambient concentrations ( $P < 0.05$ , Fig. 1, Table 2). The relationship between  $A$  and  $C_a$  was similar for 1999 and 2000 in *B. japonicus* and *S. dimidiatum* (Fig. 1A, B). For *B. ischaemum*, the slope of the relationship was similar for the 2 years, although the absolute magnitude of  $A$  was 35% lower on average in 2000 than in 1999, likely due to the severe drought in the summer of 2000 (Fig. 1C).

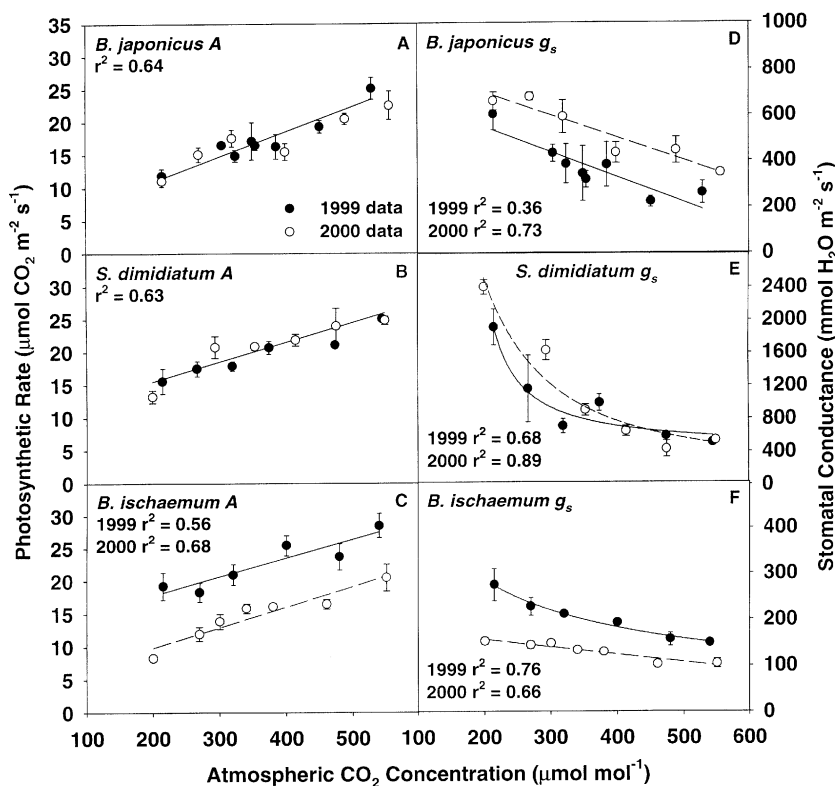
Stomatal conductance decreased significantly with increasing  $C_a$  for all species in both years (Fig. 1, Table 2). *Solanum dimidiatum* showed strong curvilinear responses on both measurement dates, with larger declines in  $g_s$  across subambient than superambient  $C_a$  (Fig. 1E). *B. ischaemum* had a curvilinear response in 1999 and a linear response in 2000 (Fig. 1F), while *B. japonicus* showed linear declines on both measurement dates (Fig. 1D). *S. dimidiatum* had the highest overall  $g_s$  of the three species in both years, followed by *B. japonicus* and *B. ischaemum*, respectively. Since  $g_s$  declined and  $A$  increased with  $CO_2$ , intrinsic leaf water-use efficiency ( $A/g_s$ ) increased linearly with  $C_a$  for all species in both years ( $P < 0.05$ , Fig. 2, Table 2). A slight curvilinear increase with  $C_a$  was observed for *S. dimidiatum* in 2000, but as this was driven largely by one outlier (shown in Fig. 2B), data were fit with a linear model.

The  $C_3$  species showed significant linear declines in the ratio of intercellular to atmospheric  $CO_2$  concentration

( $C_i/C_a$ ) with increasing  $C_a$  in both years ( $P < 0.05$ , Table 2). There was no consistent pattern in the direction of  $C_i/C_a$  changes with  $C_a$  for *B. ischaemum*.

#### Photosynthetic acclimation and light responses

There was evidence of up-regulation of  $A$  at subambient  $C_a$  for *S. dimidiatum*. The light saturated rate of carboxylation ( $V_{cmax}$ ) calculated from  $A/C_i$  curves decreased significantly with increasing  $C_a$  up to concentrations approaching current ambient (Fig. 3A, Table 3). However, superambient  $C_a$  had no effect on  $V_{cmax}$ , suggesting acclimation to high  $C_a$  did not occur for this species. The values for maximum electron transport rate ( $J_{max}$ ) for *S. dimidiatum* were variable and not significantly related to  $C_a$  concentration (Fig. 3B, Table 3). The ratio of electron transport capacity to carboxylation rate ( $J_{max}/V_{cmax}$ ) provides an indicator of N allocation to different components of the photosynthetic process in the leaf (Medlyn 1996). In *S. dimidiatum*,  $J_{max}/V_{cmax}$  increased significantly from subambient to ambient  $C_a$  and did not change over superambient  $C_a$  (Fig. 3C, Table 3), suggesting proportionally greater allocation of N to Rubisco at subambient  $C_a$ . For *B. japonicus*,  $V_{cmax}$ ,  $J_{max}$  and  $J_{max}/V_{cmax}$  were highly variable and showed no significant relationship with  $C_a$  concentration (Fig. 3D–F, Table 3).



**Fig. 1** Net photosynthetic ( $A$ ) and stomatal conductance ( $g_s$ ) rates for three species along a gradient of  $C_a$  concentrations in 1999 and 2000. Note the change in the conductance scale for the different species. Open symbols and dashed regression lines represent 2000 data. Data in panels A and B are plotted with a single line because regression results for the 1999 and 2000 data were very similar. See Table 2 for complete analysis results. Combined 1999/2000 regression results for panels A and B, respectively: parameter = 0.0308, intercept = 5.70,  $P < 0.0001$ ; parameter = 0.0279, intercept = 9.82,  $P < 0.0001$ .

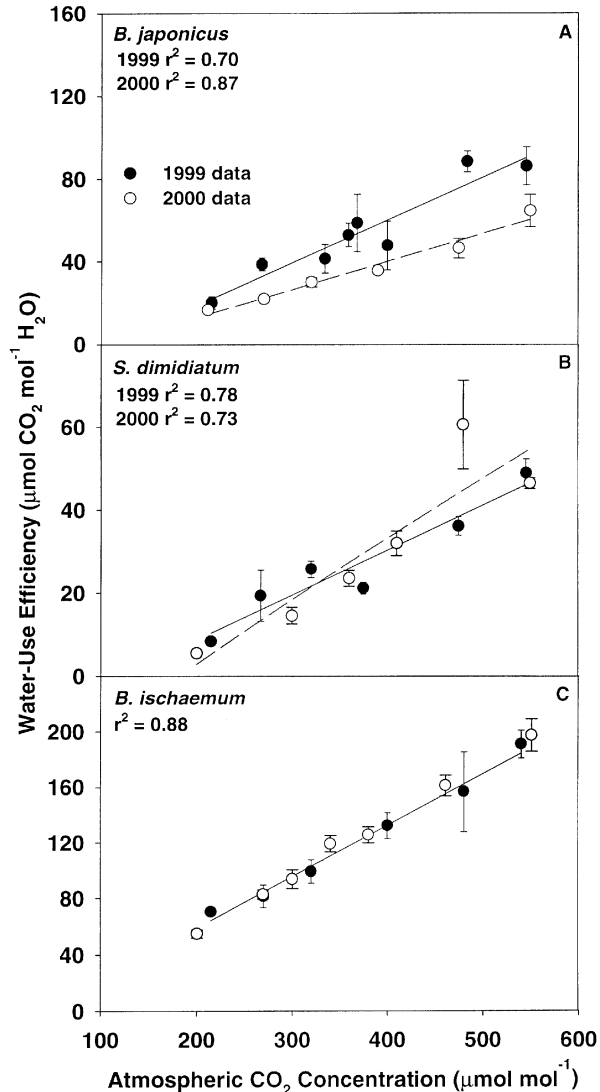


Fig. 2 Intrinsic leaf water-use efficiency ( $A/g_s$ ) for three species along a gradient of  $C_a$  concentrations in 1999 and 2000. Note the change in the  $y$ -axis scale for the different species. See Fig. 1 legend for descriptions of panels and symbols, and Table 2 for analysis results. Combined 1999/2000 regression result for panel C: parameter = 0.3911, intercept = -21.48,  $P < 0.0001$ .

Although data were variable, there was a significant decrease in PEP carboxylase efficiency with  $C_a$  concentration for *B. ischaemum* (Fig. 3G, Table 3).  $CO_2$  saturated photosynthesis ( $A_{max}$ ) did not vary significantly with  $C_a$  concentration (Fig. 3H), but plants exposed to subambient  $C_a$  concentrations generally reached  $A_{max}$  at lower  $C_i$  concentrations than plants exposed to superambient  $C_a$  (Fig. 3I).

*B. japonicus* showed a significant linear increase in apparent quantum yield with  $C_a$  (Fig. 4A, Table 3).

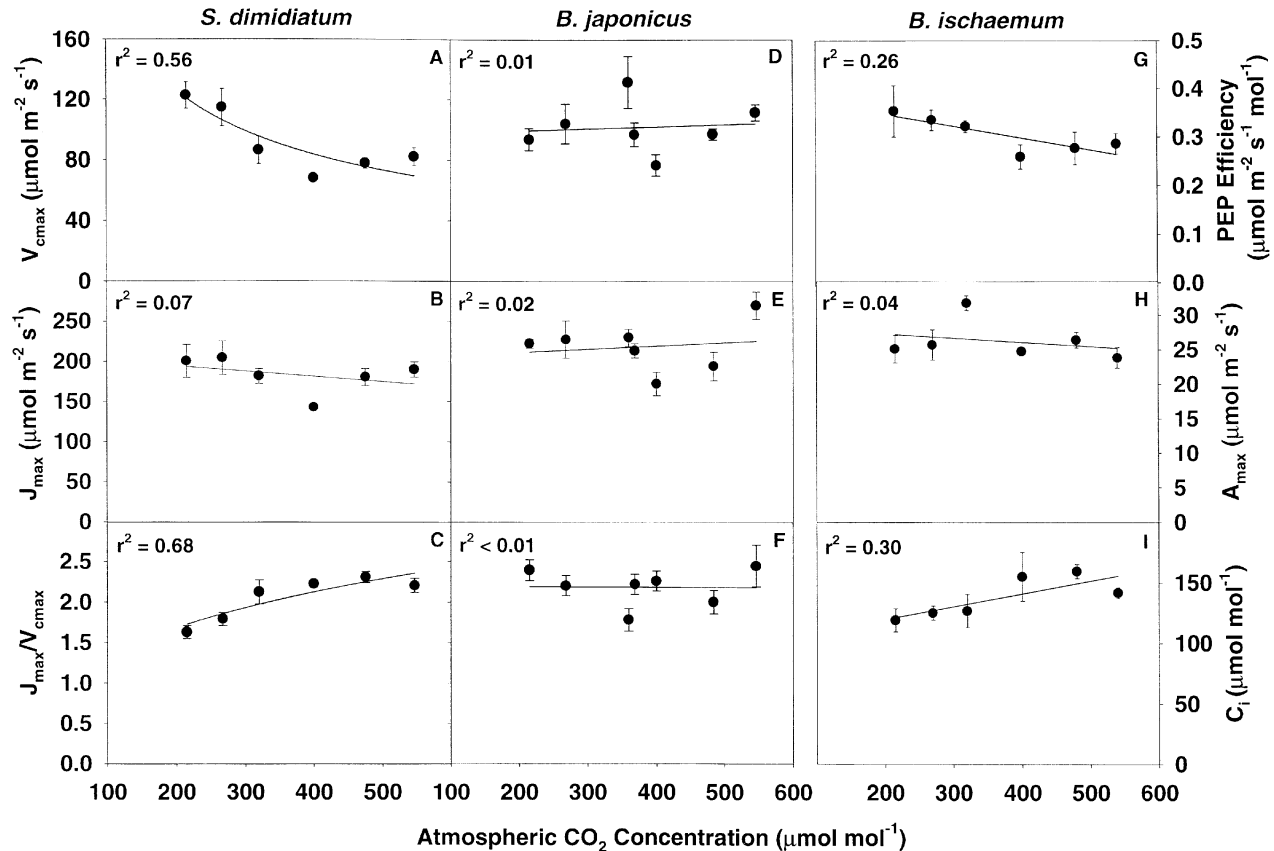
*S. dimidiatum* and *B. ischaemum* showed linear increases in quantum yield with  $C_a$  up to 320 and 400  $\mu\text{mol mol}^{-1}$ , respectively, and then the response levelled off for both species (Fig. 4B, C, Table 3). Thus, quantum yield did not change significantly over superambient  $C_a$  concentrations for either *S. dimidiatum* or *B. ischaemum*, even though superambient  $C_a$  had strong effects on light-saturated  $A$  in these same plants.

#### Leaf N and PNUE

All species showed significant or marginally significant decreases in leaf N content per unit area with increasing  $C_a$  in 1998 (Fig. 5, Table 4). Data were more variable in 1999: *B. japonicus* showed a linear decrease and there was no significant relationship between leaf N and  $CO_2$  for *B. ischaemum* or *S. dimidiatum*. As leaf N generally decreased and  $A$  increased with  $CO_2$ , PNUE increased significantly with  $CO_2$  for all species in 1999 (Fig. 6, Table 4).

#### Discussion

The goal of our study was to characterize leaf gas exchange responses of  $C_3$  and  $C_4$  grassland species to a continuous  $C_a$  gradient from past to future concentrations. One of our most important findings was an increased maximum rate of carboxylation ( $V_{cmax}$ ) at subambient  $C_a$  for the  $C_3$  perennial forb *S. dimidiatum*. To our knowledge, this is the first field study to document up-regulation of  $A$  in response to low  $C_a$ . In  $C_3$  plants, light-saturated  $A$  is limited by  $V_{cmax}$  and by the regeneration rate of RuBP. The  $V_{cmax}$  limitation occurs at low  $C_i$ , while RuBP regeneration is limited by the maximum electron transport capacity ( $J_{max}$ ) at intermediate  $C_i$ , and by inorganic phosphate  $P_i$  regeneration at high  $C_i$  (Sage 1994). It has been proposed that plants adjust N allocation among the biochemical components of  $A$  in response to changing  $C_a$ , devoting less N to carbon fixation and more to RuBP regeneration as  $C_a$  increases (Sage 1990; Sage 1994). Medlyn (1996) predicted that such N re-allocation should result in a 40% increase in  $J_{max}/V_{cmax}$  with a doubling of  $C_a$ . We found that  $J_{max}/V_{cmax}$  in *S. dimidiatum* increased by 37% as  $C_a$  increased from 200 to 400  $\mu\text{mol mol}^{-1}$  (Fig. 3, Table 3). *B. ischaemum* at subambient  $C_a$  also showed increased PEP carboxylase efficiency and reached maximum  $A$  at a lower  $C_i$ , suggesting up-regulation of  $A$  in this species as well (Fig. 3, Table 3). Assuming the activation state and specific activity of Rubisco remain constant (Sage *et al.* 1989), our data suggest that N re-allocation within the photosynthetic system does occur with  $C_a$  increases across subambient concentrations. This implies that significant photosynthetic adjustments may have taken place as  $C_a$  rose in the geological past.  $P_i$  regeneration



**Fig. 3** Photosynthetic acclimation parameters for three species along a  $C_a$  gradient in 1999: (A, D) Maximum rate of Rubisco carboxylation ( $V_{cmax}$ ) for the  $C_3$  species *S. dimidiatum* and *B. japonicus*; (B, E) maximum electron transport rate ( $J_{max}$ ) for *S. dimidiatum* and *B. japonicus*; (C, F) ratio of maximum electron transport rate to maximum carboxylation rate ( $J_{max}/V_{cmax}$ ) for *S. dimidiatum* and *B. japonicus*; (G) efficiency of the PEP carboxylase  $CO_2$  pump for the  $C_4$  grass *B. ischaemum*; (H) maximum  $CO_2$ -saturated  $A$  rate for *B. ischaemum*; and (I)  $C_i$  concentration at which  $A$  reached saturation for *B. ischaemum*. See Table 3 for analysis results.

limitations on  $A$  were not estimated in our study, but are not likely to influence  $A$  strongly at low  $C_a$  (Stitt 1991).

Our data contrast with previous studies of acclimation to subambient  $C_a$ , which did not find strong evidence for photosynthetic adjustments (Overdieck 1989; Thomas & Strain 1991; Tissue *et al.* 1995; Sage & Coleman 2001). To our knowledge, only three studies have found evidence of photosynthetic acclimation at low  $C_a$ . Sage & Reid (1992) and Cowling & Sage (1998) found that  $A$  is limited by  $P_i$  regeneration when *Phaseolus vulgaris* plants grown at subambient  $C_a$  were exposed to superambient  $C_a$ ; and Gesch *et al.* (2000) observed increased Rubisco small subunit gene expression in *Oryza sativa* L. exposed to subambient  $C_a$ . Different acclimation responses among studies could be explained in the context of carbon source-sink dynamics. Several authors have suggested that down-regulation of  $A$  at superambient  $C_a$  results from sink limitation, such as occurs when roots are restricted (Thomas & Strain 1991; reviewed by Sage 1994)

or when other resources are limiting (Curtis 1996; Bryant *et al.* 1998; but see Huxman *et al.* 1998). Exposure to subambient  $C_a$  in a field system such as ours may favour up-regulation of  $A$  in some species due to large sink demands coupled with substrate limitation of  $A$ .

Up-regulation of  $A$  may be related to increases in leaf N content, as well as to re-allocation of N within leaves. As Rubisco is a significant N investment, Sage & Reid (1992) predicted that large increases in leaf N would be needed for *P. vulgaris* grown at  $200 \mu\text{mol mol}^{-1} C_a$  to achieve  $A$  equal to plants grown at  $350 \mu\text{mol mol}^{-1}$ . We found significantly higher leaf N ( $\text{g m}^{-2}$ ) in all species at subambient  $C_a$  concentrations in 1998 (Fig. 5, Table 4), which could be additional evidence for up-regulation in *S. dimidiatum* and perhaps *B. ischaemum*. Leaf thickening in plants at high  $C_a$  could also create this trend, but no differences in leaf mass per unit area were found along the gradient for any species (L. J. Anderson and H. Maherali, unpublished data). In addition, direct meas-



**Table 3** Regression analysis results on the relationship between C<sub>a</sub> concentration and photosynthetic acclimation and light response parameters. Abbreviations are described in the legend of Table 2.

Variable, species and measurement date	Model type	Increase or decrease with C <sub>a</sub>	Parameter value (a)	Intercept (b)	r <sup>2</sup>	P-value
Photosynthetic acclimation parameters for <i>B. japonicus</i> in Apr 1999 and <i>S. dimidiatum</i> in Jul 1999						
V <sub>cmax</sub>						
<i>B. japonicus</i>	Linear	NS	0.0139	96.31	0.01	0.7619
<i>S. dimidiatum</i>	Power	Decrease	-0.5638	2420.5	0.56	0.0005
J <sub>max</sub>						
<i>B. japonicus</i>	Linear	NS	0.0410	203.28	0.02	0.5940
<i>S. dimidiatum</i>	Linear	NS	-0.05340	207.90	0.07	0.3385
J <sub>max</sub> /V <sub>cmax</sub>						
<i>B. japonicus</i>	Linear	NS	-0.00004	2.20	0.00	0.9578
<i>S. dimidiatum</i>	Power	Increase	0.3766	0.22	0.68	0.0001
Photosynthetic acclimation parameters for <i>B. ischaemum</i> in Aug 1999						
PEP carboxylase efficiency	Linear	Decrease	-0.00024	0.40	0.26	0.0320
A <sub>max</sub>	Linear	NS	-0.00611	28.53	0.04	0.4059
Internal CO <sub>2</sub> concentration	Linear	Increase	0.10574	98.56	0.30	0.0195
Quantum yield						
<i>B. japonicus</i> , Apr 2000	Linear	Increase	0.00005	0.0356	0.61	0.0001
<i>S. dimidiatum</i> , Jun 1999	Power	Increase	0.3613	0.0065	0.40	0.0091
<i>B. ischaemum</i> , Aug 1999	Power	Increase	0.5378	0.0023	0.62	0.0001

urements of soil bulk N in the experimental chambers did not show a strong underlying pattern (R. Gill, unpublished data), suggesting that leaf N levels are driven by plant and soil responses to the C<sub>a</sub> gradient itself.

We found little evidence for photosynthetic acclimation (i.e. down-regulation) at superambient C<sub>a</sub>. Photosynthesis showed a significant linear increase with C<sub>a</sub> for all species in 1999 and 2000 (Fig. 1, Table 2), indicating that sensitivity to the C<sub>a</sub> gradient persisted even after 3 years of CO<sub>2</sub> exposure. V<sub>cmax</sub> and J<sub>max</sub> parameters did not show acclimation to high C<sub>a</sub> in any species (Fig. 3, Table 3). These data are consistent with many other field studies showing no A down-regulation and no photosynthetic adjustment at superambient C<sub>a</sub> (Sage 1994; Curtis & Wang 1998; Medlyn *et al.* 1999; Herrick & Thomas 2001).

An important focus of our work was to compare plant physiological responses to increases over sub- and superambient C<sub>a</sub>. Responses were strongly linear for A, indicating that A was affected similarly by C<sub>a</sub> increases over the full range of concentrations. However, responses were often curvilinear for g<sub>s</sub>, with steeper declines over subambient C<sub>a</sub> (Fig. 1, Table 2). These data suggest that A for *B. japonicus*, *S. dimidiatum* and *B. ischaemum* may have been increasing with C<sub>a</sub> over

geological time and will likely be sensitive to future C<sub>a</sub> increases, which has important implications for biomass accumulation and ecosystem CO<sub>2</sub> fluxes in this grassland (Mielnick *et al.* 2001). However, g<sub>s</sub> was more sensitive to increases over sub- than superambient C<sub>a</sub> in the perennial species, such that future increases in C<sub>a</sub> may not influence g<sub>s</sub> as strongly as did the past rise in C<sub>a</sub>. Reductions in g<sub>s</sub> at superambient C<sub>a</sub> have been linked with increased leaf water-use efficiency, plant water potentials and soil water content (e.g. Field *et al.* 1995; H. W. Polley, unpublished data). High g<sub>s</sub> at subambient C<sub>a</sub> may have influenced these parameters in the geological past, with low C<sub>a</sub> exacerbating the effects of drought and other environmental stresses on plants (Polley *et al.* 1993; Cowling & Sage 1998; Sage & Cowling 1999). This may complicate reconstructions of past vegetation and climate patterns, which assume that species' physiological tolerances for arid conditions are similar to those observed today (Cowling & Sykes 1999).

Curvilinear responses in g<sub>s</sub> also raise questions about the C<sub>a</sub> levels to which plants are adapted. Sage & Cowling (1999) proposed that extant plants are still adapted to the low C<sub>a</sub> conditions of the past, such that their responses to future C<sub>a</sub> increases could be constrained. The relative lack of sensitivity of g<sub>s</sub> to superambient C<sub>a</sub> in our perennial species is consistent with

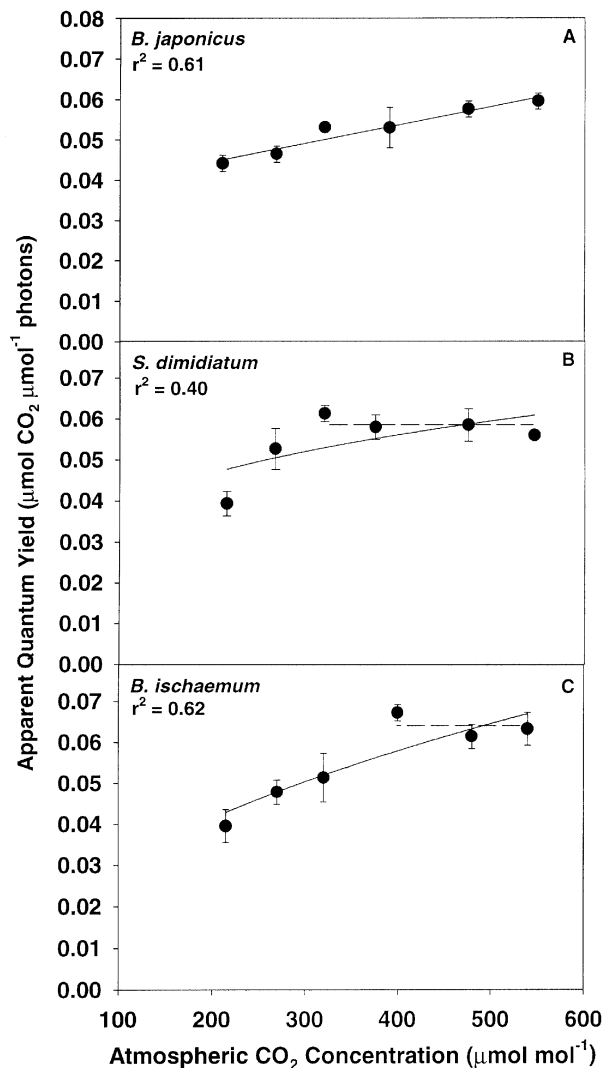
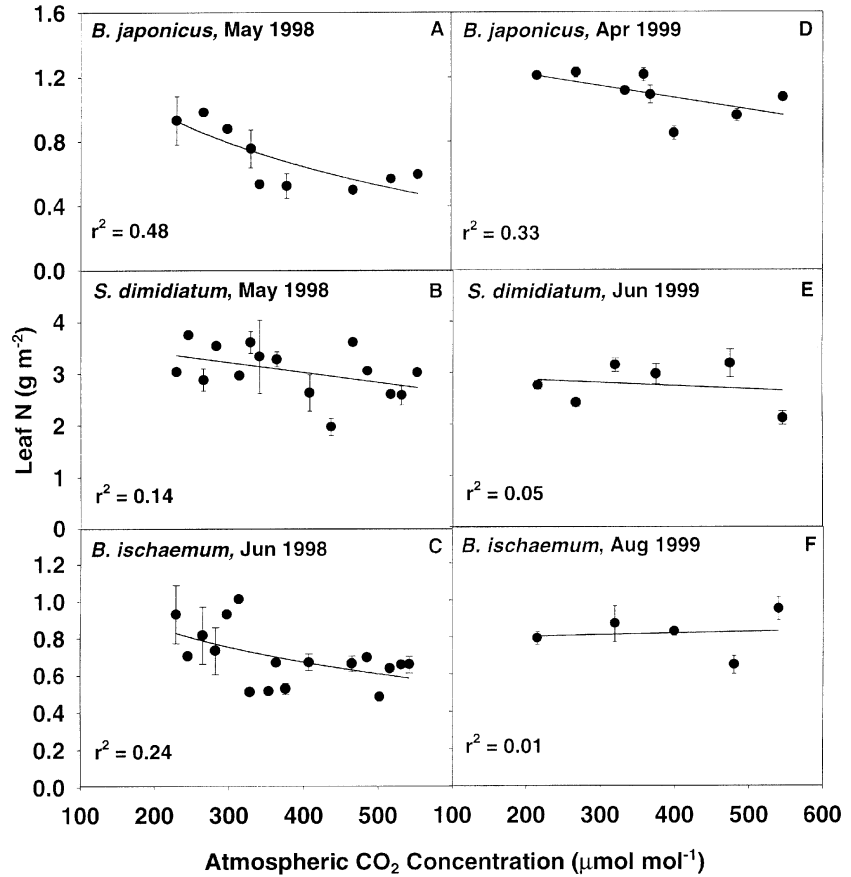


Fig. 4 Apparent quantum yields for three species along a gradient of  $C_a$  concentration in 1999 (*S. dimidiatum* and *B. ischaemum*) and 2000 (*B. japonicus*). See Table 3 for analysis results. Dashed lines in panels B and C were hand-fit to emphasize the flattening of the responses at superambient  $CO_2$  that are not well described by the power functions fit to the full data sets.

this idea, but increased  $A$  at superambient  $C_a$  and the linear response of  $g_s$  to  $C_a$  in *B. japonicus* are not. Traits vary in their sensitivity to  $C_a$  and their degree of genetic variability (e.g. Curtis *et al.* 1996; Case *et al.* 1998; Ward *et al.* 2000), such that the selective pressure of  $C_a$  on  $A$  and  $g_s$  may differ among species and genotypes. Environmental conditions may also affect the strength of  $C_a$  as an agent of selection. Occasional changes in the shape of the  $g_s$  response curve over the 2 years in this study (Fig. 1, Table 2) imply that effects of  $C_a$  on leaf physiology may be modulated by resource availability.

Like many studies at superambient  $C_a$  (reviewed in Drake *et al.* 1997), we found that increasing  $C_a$  enhanced leaf-level resource-use efficiency. The combination of decreasing leaf N and increasing  $A$  at higher  $C_a$  concentrations led to linear increases in PNUE for all species in 1999 (Figs 1, 5, 6, Table 4). Water-use efficiency also increased linearly with  $C_a$  for all three species in both years (Fig. 2, Table 2), similar to findings by Polley *et al.* (1993, 1995) for  $C_3$  plants grown in a subambient  $C_a$  gradient in greenhouse studies. Because the  $C_i/C_a$  ratio decreased with increasing  $C_a$  for the  $C_3$  species in this study (Table 2),  $A/g_s$  of  $C_3$  species increased proportionally more than did  $C_a$  ( $A/g_s = C_a(1 - C_i/C_a)/1.6$ ). For example, a 33% increase in  $C_a$  from 270 to 360  $\mu\text{mol mol}^{-1}$  resulted in a 56% increase in  $A/g_s$  for *B. japonicus* in 1999. Sage (1994) observed that  $C_i/C_a$  often decreases at superambient  $C_a$  under drought or humidity stress, and suggested that stomata will become more conservative under water stress at future  $C_a$  levels. The dry conditions in our summer field experiment may explain why we saw declines in  $C_i/C_a$ , in contrast to the near-constant  $C_i/C_a$  ratios observed across  $C_a$  concentrations in several greenhouse studies (e.g. Sage & Reid 1992; Polley *et al.* 1993; Sage 1994; but see Tissue *et al.* 1995; Santrucek & Sage 1996). Many factors affect scaling of resource use efficiencies from leaves to canopies (Field *et al.* 1995), but the strong enhancements in PNUE and  $A/g_s$  observed here indicate the importance of past and future increases in  $C_a$  for productivity and resource balance of grasslands.

Leaf gas exchange data were also collected for the three study species along the  $C_a$  gradient in 1998, using the LI-6200 closed gas exchange system and ambient light (Li-Cor, Inc., Lincoln, NE, USA). The LI-6200 is inferior to the LI-6400 in its control of light, temperature and humidity in the leaf chamber, yet 1998 gas exchange trends were generally consistent with those seen in 1999/2000. In 1998, all species showed significant, generally curvilinear decreases in  $g_s$  with increasing  $C_a$ , as in 1999 and 2000 ( $P < 0.01$  for all, data not shown). The  $C_3$  species also showed increases in PNUE with  $C_a$  in May 1998 ( $P < 0.05$ , data not shown). One difference between 1998 and 1999/2000 was a less dramatic  $A$  response to increasing  $C_a$  for *S. dimidiatum* and *B. ischaemum*. Photosynthesis increased with  $C_a$  for both species, but the data were more variable than in 1999/2000, and not statistically significant. This may reflect the poorer environmental control of LI-6200 compared to the LI-6400, or the fact that measurement light levels in 1998 were well below saturation for the perennial species ( $\geq 1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ , see also Table 1). Non-saturating light levels can reduce responses to  $C_a$ , particularly in  $C_4$  plants (Sionit & Patterson 1984). Despite the reduced  $A$  response in 1998,  $A/g_s$  increased linearly with increasing  $C_a$  for all three species ( $P < 0.01$ , data not shown).



**Fig. 5** Leaf N content for three species along a gradient of C<sub>a</sub> concentration in 1998 and 1999. Note the change in the y-axis scale for the different species. See Table 4 for analysis results.

**Table 4** Results of regression analyses on the relationship between C<sub>a</sub> concentration and leaf N content for three species in 1998 and 1999 and photosynthetic nitrogen-use efficiency for three species in 1999. Abbreviations are described in the legend of Table 2.

Variable, species and measurement date P-value	Model type	Increase or decrease with C <sub>a</sub>	Parameter value(s)	Intercept (a)	r <sup>2</sup> (b)	P-value
<b>Leaf N content (g m<sup>-2</sup>)</b>						
<i>B. japonicus</i> , May 1998	Log	Decrease	-0.4771	3.50	0.48	0.0060
<i>B. japonicus</i> , Apr 1999	Linear	Decrease	-0.0008	1.39	0.33	0.0062
<i>S. dimidiatum</i> , May 1998	Linear	NS	-0.0023	3.89	0.14	0.0539
<i>S. dimidiatum</i> , Oct 1998	Linear	Decrease	-0.0020	3.59	0.18	0.0418
<i>S. dimidiatum</i> , Jun 1999	Linear	NS	-0.0008	3.03	0.05	0.4082
<i>B. ischaemum</i> , Jun 1998	Log	Decrease	-0.2904	2.41	0.24	0.0071
<i>B. ischaemum</i> , Sep 1998	Linear	Decrease	-0.0009	1.17	0.19	0.0175
<i>B. ischaemum</i> , Aug 1999	Linear	NS	0.00009	0.78	0.01	0.7812
<b>Photosynthetic nitrogen-use efficiency (A/leaf N content)</b>						
<i>B. japonicus</i> , Apr 1999	Linear	Increase	0.0391	1.39	0.78	< 0.0001
<i>S. dimidiatum</i> , Jun 1999	Linear	Increase	0.0144	2.14	0.55	0.0007
<i>B. ischaemum</i> , Aug 1999	Linear	Increase	0.0305	17.24	0.46	0.0054

Therefore, increases in A/g<sub>s</sub> in 1998 were largely driven by changes in g<sub>s</sub> along the C<sub>a</sub> gradient, suggesting that under non-saturating light conditions (which occur

frequently in the field), A/g<sub>s</sub> responses to increasing C<sub>a</sub> may be mediated largely by stomatal closure, not A enhancement.

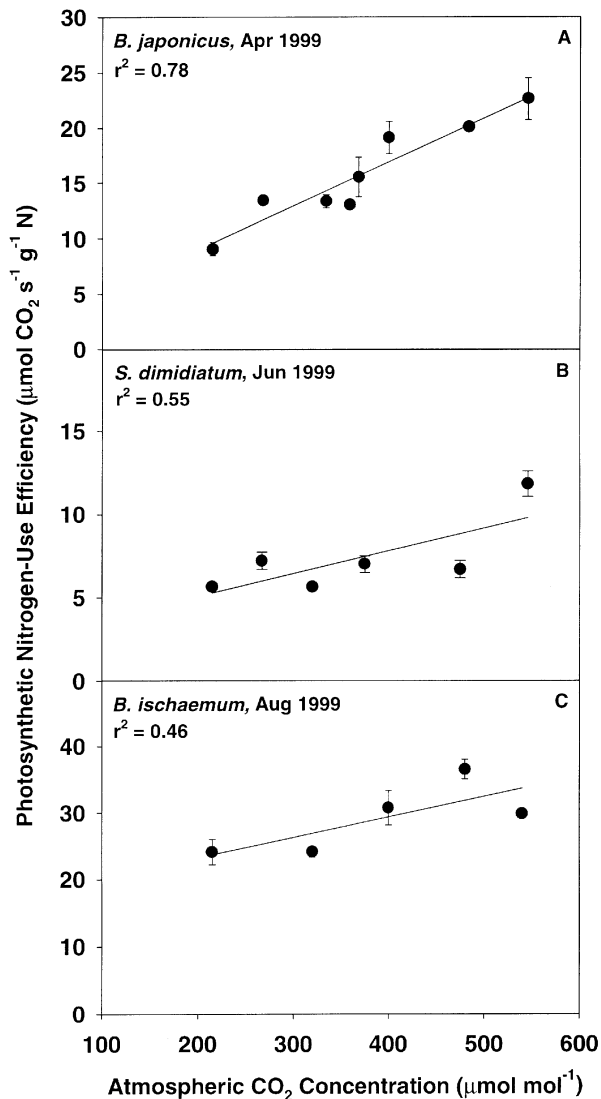


Fig. 6 Photosynthetic nitrogen-use efficiency ( $A/\text{leaf N}$  content) for three species along a gradient of  $C_a$  concentration in 1999. Note the change in the  $y$ -axis scale for the different species. See Table 4 for analysis results.

Both the  $C_3$  and  $C_4$  species in this study responded to changes in  $C_a$ . Increasing  $C_a$  enhanced  $A$  slightly more in the  $C_3$  than the  $C_4$  species, yet  $A$ ,  $A/g_s$  and PNUe increased, and  $g_s$  and leaf N decreased, significantly with increasing  $C_a$  for the  $C_4$  grass *B. ischaemum* (Figs 1, 2, 5, 6). Others have also shown that  $C_4$  grassland species respond to increased  $C_a$  with enhanced water-use efficiency and growth, and changes in tissue nutrient concentrations (e.g. Knapp *et al.* 1993; Owensby *et al.* 1993; Ghannoum *et al.* 2000). Decreases in PEP carboxylase content, bundle sheath cell wall thickness (Watling *et al.* 2000) and

$A_{\text{max}}$  (Morgan *et al.* 1994) have also been found in  $C_4$  plants at high  $C_a$ , suggesting that  $C_4$  species undergo photosynthetic acclimation. Our data add to a growing new perspective on the influences of  $C_a$  on  $C_4$  plants. In a recent review, Wand *et al.* (1999) concluded that  $C_4$  plants showed significant and consistent responses to  $C_a$ , often of a similar direction and magnitude to  $C_3$  plants. Thus, rising  $C_a$  may not shift the competitive balance in favour of  $C_3$  over  $C_4$  plants to the extent once predicted.

Quantum yield has been suggested as a determinant of the relative dominance of  $C_3$  and  $C_4$  plants globally (e.g. Johnson *et al.* 1993; Ehleringer *et al.* 1997). The greater quantum yields of  $C_4$  plants at high temperatures may allow them to dominate warm sites currently, while their  $\text{CO}_2$  concentrating mechanism conferred a competitive advantage over  $C_3$  plants at low  $C_a$  levels in the past (Ehleringer *et al.* 1997).  $C_a$  mediated increases in the quantum yield of  $C_3$  plants (via suppression of photorespiration, Long & Drake 1991) are expected to favour  $C_3$  over  $C_4$  communities in the future. We found that quantum yield increased linearly with  $C_a$  across sub- and superambient concentrations for *B. japonicus*, but increased only over subambient concentrations for the other species. The lack of response in quantum yield to superambient  $C_a$  is surprising for the  $C_3$  *S. dimidiatum*, although a similar pattern was found for *S. melonghena* by Bunce & Ziska (1999), who suggested that acclimation to high  $C_a$  can reduce quantum yield. Moreover, quantum yield did not differ between  $C_3$  and  $C_4$  species at a given  $C_a$  concentration, suggesting that at current temperatures, future  $C_a$  increases may not favour  $C_3$  relative to  $C_4$  plants in this grassland. Because the three species were measured on different dates (Table 1), our results may be affected by environmental conditions.

Physiologically based models are needed to improve our predictions of ecosystem responses to past and future  $C_a$  changes, but field tests of the relationships used in these models are often difficult, particularly for past  $C_a$  concentrations. We examined plant physiological responses over a gradient of  $C_a$  from past to predicted future levels, and found that responses are not necessarily linear. In addition, even tightly coupled responses, such as  $A$  and  $g_s$ , can have different response curves. We also found that acclimation to subambient  $C_a$  may modulate the slopes of these responses for some species, and that  $C_3$  and  $C_4$  plants are both strongly affected by  $C_a$  increases. These data emphasize the need to consider plant responses in natural communities over sub- through superambient  $C_a$  in order to scale effectively from leaves to ecosystems in past and future environments.

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