

Will photosynthesis of maize (*Zea mays*) in the US Corn Belt increase in future [CO₂] rich atmospheres? An analysis of diurnal courses of CO₂ uptake under free-air concentration enrichment (FACE)

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Abstract

The C₄ grass *Zea mays* (maize or corn) is the third most important food crop globally in terms of production and demand is predicted to increase 45% from 1997 to 2020. However, the effects of rising [CO₂] upon C₄ plants, and *Z. mays* specifically, are not sufficiently understood to allow accurate predictions of future crop production. A rainfed, field experiment utilizing free-air concentration enrichment (FACE) technology in the primary area of global corn production (US Corn Belt) was undertaken to determine the effects of elevated [CO₂] on corn. FACE technology allows experimental treatments to be imposed upon a complete soil–plant–atmosphere continuum with none of the effects of experimental enclosures on plant microclimate. Crop performance was compared at ambient [CO₂] (354 μmol mol⁻¹) and the elevated [CO₂] (549 μmol mol⁻¹) predicted for 2050. Previous laboratory studies suggest that under favorable growing conditions C₄ photosynthesis is not typically enhanced by elevated [CO₂]. However, stomatal conductance and transpiration are decreased, which can indirectly increase photosynthesis in dry climates. Given the deep soils and relatively high rainfall of the US Corn Belt, it was predicted that photosynthesis would not be enhanced by elevated [CO₂]. The diurnal course of gas exchange of upper canopy leaves was measured *in situ* across the growing season of 2002. Contrary to the prediction, growth at elevated [CO₂] significantly increased leaf photosynthetic CO₂ uptake rate (*A*) by up to 41%, and 10% on average. Greater *A* was associated with greater intercellular [CO₂], lower stomatal conductance and lower transpiration. Summer rainfall during 2002 was very close to the 50-year average for this site, indicating that the year was not atypical or a drought year. The results call for a reassessment of the established view that C₄ photosynthesis is insensitive to elevated [CO₂] under favorable growing conditions and that the production potential of corn in the US Corn Belt will not be affected by the global rise in [CO₂].

Keywords: atmospheric change, climate change, drought, elevated CO₂, photosynthetic carbon gain, SoyFACE.

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Introduction

Atmospheric [CO₂] is predicted to increase to ~550 μmol mol⁻¹ by 2050, and probably exceed 700 μmol mol⁻¹ by the end of this century (Houghton *et al.*, 2001). The C₄ grass *Zea mays* L. (maize or corn) is

the third most important food crop globally in terms of production and demand is predicted to increase 45% from 1997 to 2020 (Young & Long, 2000; Rosegrant *et al.*, 2001). Therefore, any effects of elevated [CO₂] on crop productivity will have significant economic and social consequences. Nonetheless, the effects of elevated [CO₂] upon C₄ plants, and *Z. mays* specifically, are not sufficiently understood to allow accurate predictions of future crop production or to allow varietal selection for

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productivity in a changing climate (Wang & Erda, 1996; Young & Long, 2000). In particular, there are currently no experimental data on the response of *Z. mays* to growth at elevated [CO₂] under standard agricultural practice in the field. These are necessary to validate the predictions of models currently used to predict crop yields and global food security for the middle of this century (e.g. Brown & Rosenberg, 1999; Alexandrov & Hoogenboom, 2000).

Theoretically, increasing atmospheric [CO₂] will have little direct effect on photosynthesis in C₄ plants due to the dicarboxylate cycle, which maintains [CO₂] of around 10–20 times that of the current atmosphere at the active site of Rubisco in the bundle sheath cells (Furbank *et al.*, 1989; Jenkins *et al.*, 1989). However, there is an inconsistency between experimental studies observing no response (e.g. Hocking & Meyer, 1991; Ziska *et al.*, 1991) and others describing significant effects upon photosynthesis and growth at elevated [CO₂] (Knapp *et al.*, 1993; Amthor *et al.*, 1994; Poorter *et al.*, 1996; Wand *et al.*, 1999; Anderson *et al.*, 2001). Greater photosynthesis of C₄ plants at elevated [CO₂] has been variously attributed to a range of processes including: (1) direct effects on Rubisco CO₂ saturation (Wong, 1979; Watling & Press, 1997; Ziska & Bunce, 1997); (2) bundle sheath leakiness (Saliendra *et al.*, 1996; Watling *et al.*, 2000); (3) direct CO₂ fixation in the bundle sheath (Moore *et al.*, 1986); (4) C₃-like photosynthesis in immature C₄ leaves (Dai *et al.*, 1995; Ziska *et al.*, 1999); as well as (5) lower stomatal conductance under elevated [CO₂] leading to conservation of water resources and improved plant water status (Samarakoon & Gifford, 1996; Ghannoum *et al.*, 2000; Wall *et al.*, 2001). Improved water status at elevated [CO₂] appears to be the basis of the greatest observed enhancements in C₄ photosynthesis and growth, but requires that water availability and/or evaporative demand be limiting under ambient [CO₂] (Seneweera *et al.*, 1998; Ghannoum *et al.*, 2000). For instance, in enclosure studies 50% greater growth of *Z. mays* was observed when plants were drought stressed, compared with no CO₂ effect under high water availability (Samarakoon & Gifford, 1996). The response of *Z. mays* to elevated [CO₂] is therefore likely to vary among the major areas of production across temperate and tropical climatic zones (Sage & Kubien, 2003).

This study aimed to determine the effect of free-air concentration enrichment (FACE) of [CO₂] upon leaf gas exchange of field grown *Z. mays* under standard agricultural practice in Central Illinois, USA. FACE technology allows experimental treatments to be imposed upon a complete soil–plant–atmosphere continuum with none of the effects of experimental enclosures on plant microclimate (Hendrey *et al.*, 1993;

McLeod & Long, 1999). The Midwest Corn Belt of the USA, where growing conditions are very favorable (mean summer monthly rainfall of 95 mm), accounts for 20% of global production of *Z. mays*. A rainfed, field experiment utilizing FACE technology in the primary area of global *Z. mays* production provides a unique opportunity to determine the effects of elevated [CO₂] on *Z. mays*, with direct relevance to future agricultural practice and productivity.

Given the relatively high rainfall of Central Illinois and the absence of irrigation in typical agricultural practice of the region, *Z. mays* probably grows under sufficient water availability in a 'nondrought' year. Therefore, it was predicted that growth at elevated [CO₂] would not increase leaf photosynthesis. This paper reports the diurnal course of leaf level gas exchange of *Z. mays* grown under ambient and elevated [CO₂] at five stages of ontogenetic development. Variation in photosynthetic carbon gain over the growing season is discussed in relation to micrometeorological conditions and leaf physiology. Finally, to elucidate what the response of *Z. mays* may be in a 'typical' year the experimental conditions in 2002 are related to local long-term patterns of rainfall.

Materials and methods

Field site, cultivation and FACE system

The study was conducted at the SOYbean FACE (SoyFACE) facility in Champaign, IL, USA (40°02'N, 88°14'W, 228 m above sea level; <http://www.soyface.uiuc.edu>). The SoyFACE facility is situated on 32 ha of Illinois farmland. *Z. mays* and *Glycine max* are each planted over 16 ha. The crops are rotated between the eastern and western halves of the field on a yearly basis. The very deep Flanagan/Drummer soil series is typical of northern and central Illinois wet, dark-colored 'prairie soils' (fine silty, mixed, mesic Typic Endoaquoll). It is an organically rich, highly productive Corn Belt soil. The field is tile-drained and has been in continuous cultivation to arable crops for over 100 years.

In 2002, *Z. mays* was planted on the western half of the field (Fig. 1). Cultural practices were typical for this region of Illinois. The *Z. mays* crop received 202 kg N ha⁻¹ (157 kg ha⁻¹ as 28% 1:1 urea: ammonium nitrate liquid preplant and 45 kg ha⁻¹ credit from previous soybean N₂ fixation). *Z. mays* cv 34B43 (Pioneer Hi-Bred International, Des Moines, IA, USA) was planted on May 30 at a row spacing of 0.76 m (seed density 74 100 ha⁻¹). Emergence was on June 4 and final harvest on October 10. To control for topographic (<1 m) and soil variation, the half of the field planted to *Z. mays* was divided into four experimental blocks, each

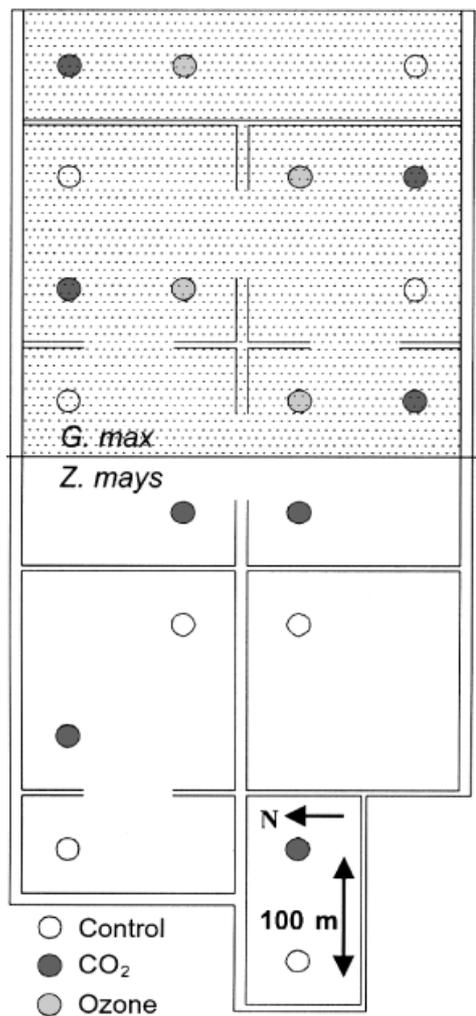


Fig. 1 Map of SOYbean Free-Air Concentration Enrichment (SoyFACE) experimental site in 2002. A 32.4 ha field growing *Glycine max* and *Zea mays*, near Urbana, IL. Experimental treatments of ambient (control) atmospheric conditions, elevated [CO₂] and elevated [ozone] are imposed within FACE rings. In 2002, corn was planted on the western 16 ha of the field. Leaf gas exchange was compared for plants growing in plots at ambient and elevated [CO₂].

containing two 20-m diameter circular plots (Fig. 1). One plot in each block was maintained at current ambient [CO₂] of 354 $\mu\text{mol mol}^{-1}$. The second plot in each block was fumigated during daylight hours, from crop planting until harvest, to an elevated [CO₂] of 549 $\mu\text{mol mol}^{-1}$ using a FACE system (Miglietta *et al.*, 2001). Briefly, this system consisted of a ring of horizontal pipes, which release CO₂ into the wind on the upwind side of the plot through submillimeter laser drilled holes along the pipe. The position and rate of release is governed by wind direction and wind speed, which is continually measured in each plot. Actual CO₂ concentration is measured and the signal used to

continuously adjust rate of release. The pipes were placed at 10 cm above the height of the crop, and were raised weekly to keep pace with growth. When the crop exceeded 1.5 m a second set of pipes were added midway between the top of the crop and the ground, to ensure enrichment through the canopy. The same structure of pipes surrounded the control plots, but no CO₂ was added. Each plot was separated by 100 m to avoid cross-contamination. The target elevated [CO₂] was 550 $\mu\text{mol mol}^{-1}$, as projected for the year 2050 by the Intergovernmental Panel on Climate Change (Houghton *et al.*, 2001). The average [CO₂] over the season was 354 $\mu\text{mol mol}^{-1}$ in ambient rings and 549 $\mu\text{mol mol}^{-1}$ in elevated rings. The [CO₂] in the elevated plots were within $\pm 20\%$ of the target for 90% of time based on 1-min averages.

Meteorological data

An on-site weather station (MetData 1-type, Cambell Scientific Ltd, Logan, UT, USA) measured air temperature (T_{air}) and relative humidity at a height of 3 m. A quantum sensor (Model QSO, Apogee Instruments, Logan, UT, USA) measured incident photosynthetic photon flux density (PPFD) at a height of 3 m. Data were averaged and logged at 10-min intervals throughout the growing season. Tipping bucket rain gauges (Model 52202, R. M. Young Inc., Traverse City, MI, USA) were distributed throughout the field and recorded rainfall events in 0.0001 m increments throughout the season.

To determine how rainfall in the 2002 growing season compared with long-term patterns, monthly rainfall data were obtained from the Illinois State Water Survey weather station in Urbana (40°05'N 88°14'W) for the period 1889–2002 (<http://www.sws.uiuc.edu/data/climate/db/>). It is situated 3 km north of the SoyFACE site and at the same altitude. The total summer rainfall for each year was calculated as the sum of rainfall in June, July and August. As a measure of the variability in rainfall, the coefficient of variance (CV) among the three, individual monthly totals was also calculated for each year.

Gas exchange

The diurnal course of gas exchange of the youngest fully expanded leaf of *Z. mays* in each ring was measured, using an open gas-exchange system incorporating open-path IR CO₂ and water vapor analyzers, and a 6 cm² leaf chamber (LI-6400; LI-COR, Inc., Lincoln, NE, USA), on five dates across the growing season. The five dates chosen corresponded to five discrete stages of crop vegetative and reproductive development (Table 1). Crop ontogenetic development was monitored by recording the vegetative and

Table 1 Calendar date and day of year (DOY) of experimental measurements with corresponding crop growth stage described as days after emergence (DAE), ontogenetic developmental stage (defined in Ritchie *et al.*, 1993) and height for *Zea mays* grown under ambient (370 $\mu\text{mol mol}^{-1}$) and elevated $[\text{CO}_2]$ (550 $\mu\text{mol mol}^{-1}$) during 2002 at SoyFACE, Urbana, IL

Date	DOY	DAE	Developmental Stage		Height (m)	
			Ambient	Elevated	Ambient	Elevated
July 11	192	37	9th leaf	9th leaf	0.79	0.83
July 22	203	48	17th leaf	17th leaf	2.06	2.38
August 9	221	66	Milky kernel	Milky kernel	2.27	2.50
August 21	233	78	Doughy kernel	Doughy kernel	2.27	2.51
September 5	248	93	Dented kernel	Dented kernel	2.28	2.51

reproductive growth stage (defined by Ritchie *et al.*, 1993) of six individuals per plot every 2 days. Two systems were used on each measurement date and calibrated against a standard known concentration of CO_2 in air (21.4% O_2 /balance N_2 ; CO_2 503 $\mu\text{mol mol}^{-1}$; S. J. Smith Welding Supply, Decatur, IL, USA) and against known humidities provided by a water vapor generator (LI-610, LI-COR, Inc.).

Measurements were at intervals of ~ 2 h from predawn to postsunset. At each time point, measurements were made in all four experimental blocks. Within each experimental block, one gas-exchange system was operated in the ambient $[\text{CO}_2]$ plot, while the other simultaneously operated in the elevated $[\text{CO}_2]$ plot. Each gas-exchange system alternately operated in ambient $[\text{CO}_2]$ and then elevated $[\text{CO}_2]$ plots while moving from block to block, during each time point. Four plants were sampled near to the center of each plot at each time interval. These procedures ensured that measurements were not confounded with variation in microclimate with time and that undetected differences between gas-exchange systems were not confounded with treatments. To avoid pseudoreplication, statistical comparisons were made using the mean of the four plants measured in each plot as sample value for the plot. Sample size was therefore $n = 4$; representing the four blocks. Measurements on all individuals were made at growth $[\text{CO}_2]$. PPFD and T_{air} were recorded at the top of the canopy prior to starting the sampling procedure at each time point. Then throughout that time point the conditions were replicated in each leaf chamber by the red-blue LED light source and Peltier heat sinks. The water vapor concentration of air entering the chamber was not controlled and therefore tracked ambient conditions. Data were collected once the CV of infra red gas analyser (IRGA) output was below 0.7%. Leaf net CO_2 assimilation (A), stomatal conductance (g_s) and $[\text{CO}_2]$ of the intercellular (substomatal) air space (c_i) were calculated using the equations of von Caemmerer & Farquhar (1981). Estimates of g_s at dawn and dusk were unreliable due

to the formation of dew on leaves and are therefore not reported. Transpiration, i.e. water vapor efflux per unit leaf area (E), measured by the gas-exchange system is a function of chamber humidity, which may differ from that of the external atmosphere. A better measure of transpiration was therefore the product of leaf conductance and the leaf vapor pressure deficit, derived from measured leaf temperature and the external ambient air humidity. Daily photosynthetic carbon gain (A') by the upper canopy leaves was calculated by fitting a fifth or sixth order polynomial to the diurnal course of A and integrating under this curve over the period for which the light-compensation point of photosynthesis was exceeded.

Statistical analysis

For the overall comparison of A and g_s between CO_2 treatments over the diurnal period, a mixed model was fitted to repeated measures in time. Time of day (measurement period) and CO_2 treatment were assessed to be fixed effects, while block was a random effect. For the overall comparison of A' , c_i , c_i/c_a and E between treatments over the growing season, a mixed model was fitted to repeated measures of time. Day of year (measurement period) and CO_2 treatment were assessed to be fixed effects, while block was a random effect. In all cases, statistics were performed on plot means using the MIXED procedure in SAS, with the Kenwood-Rogers option (SAS Institute, Cary, NC, USA). The Akaike's criterion was used to choose the best model of variance-covariance. Individual tests used one of the following models: various components heterogeneous autoregressive; various components autoregressive; heterogeneous autoregressive; various components heterogeneous compound symmetry; and autoregressive. In all cases, pairwise comparisons of estimated means within measurement periods were planned *a priori*, and obtained using the paired differences of means (PDIFF) option on the least-squares means (LSMEANS) statement (SAS Institute;

Steel *et al.*, 1997). In addition, *a posteriori* contrasts, adjusted by Bonferroni, were used to compare *A* at 10:30 and 12:45 hours on August 9 under both ambient and elevated [CO₂]. The progression of crop ontogenetic development over the season was compared under ambient and elevated [CO₂] using a general linear model (proc GLM; SAS Institute). Preplanned pairwise comparisons of development between treatments on each measurement date were made using Tukey's tests ($P < 0.05$).

Results

Diurnal patterns of A, g_s and microclimate

Microclimatic conditions on the dates of gas-exchange measurements varied within the typical range for summer months in the Midwest USA with daily peak values of: PPFD 1900–2175 $\mu\text{mol m}^{-2} \text{s}^{-1}$; T_{air} 27–33 °C;

and air saturation deficit (*D*) 1.5–2.5 kPa (Fig. 2). The sky was predominantly clear on all days except July 11 when heavy afternoon cloud cover reduced total daily PPFD.

Under both ambient and elevated [CO₂], the diurnal course of *A* corresponded generally with the variation in PPFD over each daily period (Figs 2 and 3). At times, *A* was up to 41% greater in leaves grown and measured under elevated [CO₂] compared with ambient [CO₂] (Fig. 3). Early in the season, growth under elevated [CO₂] resulted in continuously greater *A* for a significant fraction of the day (Fig. 3; July 11: $F_{1,3} = 40.36$, $P = 0.008$; July 22: $F_{1,3} = 5.85$, $P = 0.029$). Midseason, on August 9, there was no overall effect of elevated [CO₂] on *A* (Fig. 3; $F_{1,3} = 1.50$, $P = 0.308$). However, there was a midday depression of *A* between 10:30 and 12:45 hours. This reduction in *A* was large and statistically significant under ambient [CO₂] (–25%; $df = 9$, $t = 3.97$, $P = 0.016$), but not under elevated [CO₂] (–9%; $df = 9$, $t = 1.02$, $P = 0.334$). By the end of the season, there was

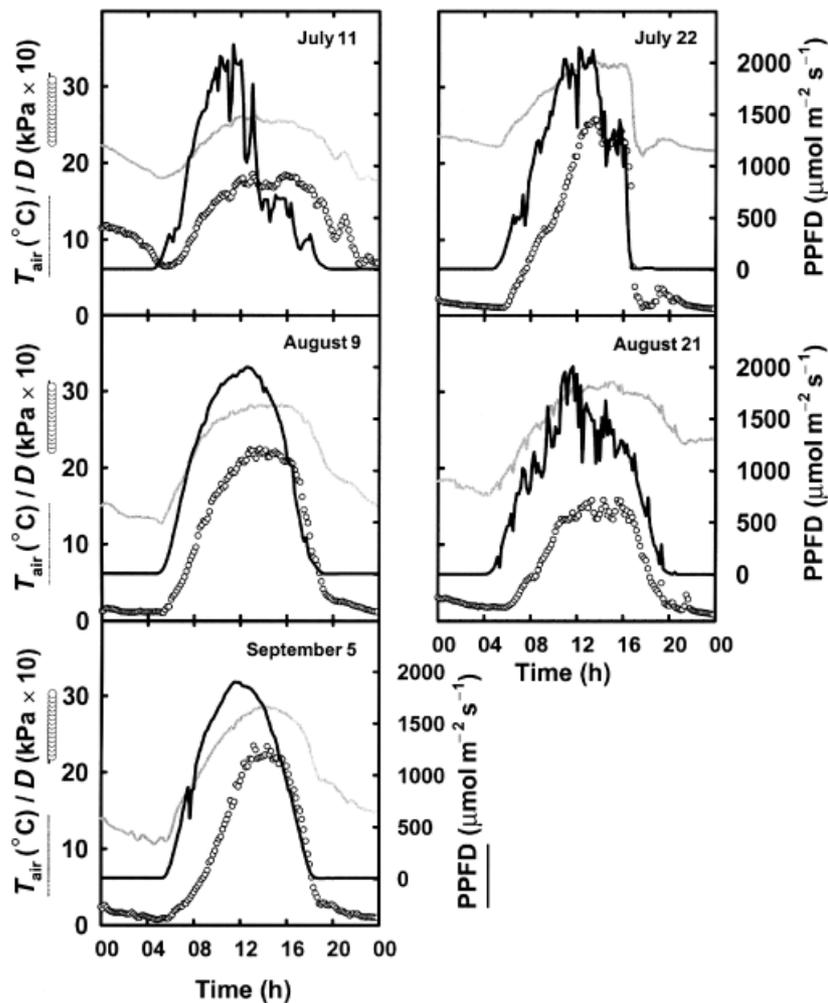


Fig. 2 Diel traces of photosynthetic photon flux density (PPFD; $\mu\text{mol m}^{-2} \text{s}^{-1}$), air temperature (T_{air} ; °C) and air saturation deficit (*D*; $\text{kPa} \times 10$) on five dates of gas exchange measurements on *Zea mays* during 2002 at SoyFACE, Urbana, IL.

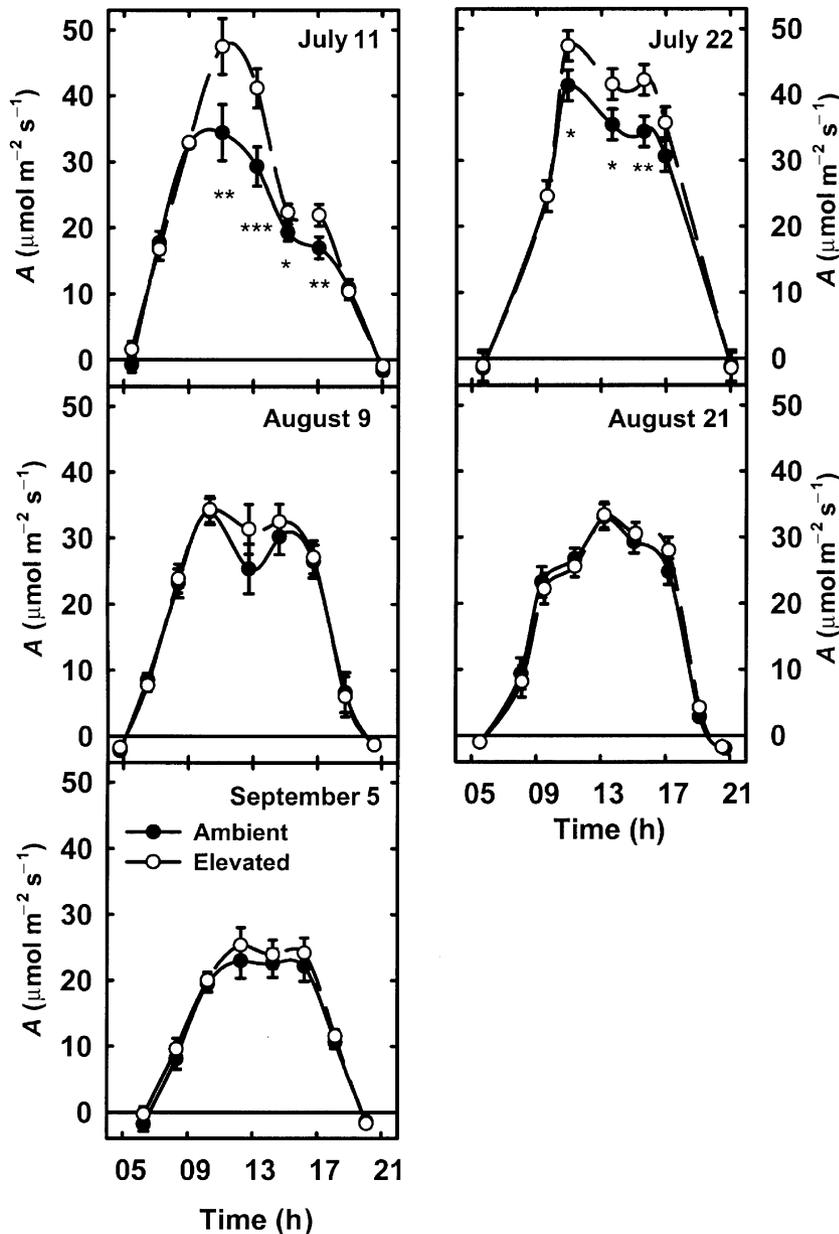


Fig. 3 Diurnal courses of net photosynthetic CO_2 assimilation (A ; $\mu\text{mol m}^{-2} \text{s}^{-1}$) of the youngest fully expanded leaf of *Zea mays* grown under ambient ($370 \mu\text{mol mol}^{-1}$; \bullet) and elevated [CO_2] ($550 \mu\text{mol mol}^{-1}$; \circ) on five dates during 2002 at SoyFACE, Urbana, IL. Each point is the mean (\pm SE) of the replicate plots measured at that time ($n = 4$). Statistically significant differences between treatments within measurement periods are indicated by asterisks: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

consistently no significant effect of elevated [CO_2] on A (August 21: $F_{1,3} = 1.11$, $P = 0.370$; September 5: $F_{1,3} = 3.50$, $P = 0.158$).

The response of g_s to elevated [CO_2] varied significantly with the date. On July 11 there was no significant effect of elevated [CO_2] on stomatal conductance (Fig. 4; $F_{1,3} = 1.74$, $P = 0.279$). However, g_s was significantly lower (-23% on average) under elevated [CO_2], compared with ambient [CO_2], on all measurement dates

later in the season (Fig. 4; July 22: $F_{1,3} = 21.59$, $P = 0.029$; August 9: $F_{1,3} = 20.17$, $P = 0.021$; August 21: $F_{1,3} = 33.11$, $P = 0.010$; September 5: $F_{1,3} = 11.61$, $P = 0.042$).

Seasonal patterns of crop ontogenetic development, A' and rainfall

There was no significant difference in the progression of either vegetative or reproductive crop ontogenetic

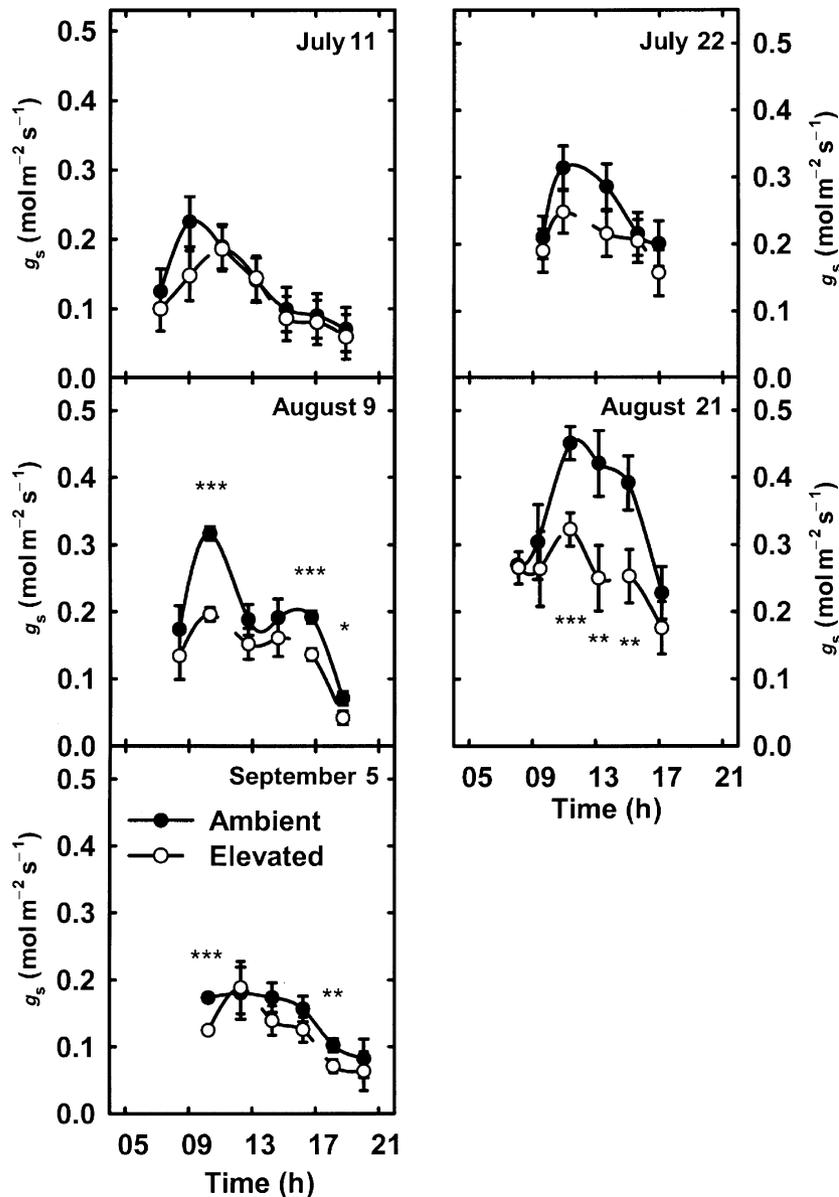


Fig. 4 Diurnal courses of stomatal conductance (g_s ; $\text{mol m}^{-2} \text{s}^{-1}$) of the youngest fully expanded leaf of *Zea mays* grown under ambient ($370 \mu\text{mol mol}^{-1}$; \bullet) and elevated [CO_2] ($550 \mu\text{mol mol}^{-1}$; \circ) on five dates during 2002 at SoyFACE, Urbana, IL. Each point is the mean (\pm SE) of the replicate plots measured at that time ($n = 4$). Statistically significant differences between treatments within measurement periods are indicated by asterisks: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

development under ambient and elevated [CO_2] (Fig. 5). Under elevated [CO_2], the seasonal average of A' was significantly greater (10%) than under ambient [CO_2] (Fig. 6; $F_{1,6} = 21.02$, $P = 0.004$). However, there was a significant interaction between this treatment effect and the date ($F_{4,24} = 3.41$, $P = 0.024$). An early season enhancement of A' under elevated [CO_2] by 21% on July 11 and 11% on July 22 corresponded to a period of low rainfall (Fig. 6). Total rainfall was 0.1 and 21.9 mm in the 2 weeks prior to July 11 and 22, respectively. In contrast, there was no significant

difference between A' under ambient and elevated [CO_2] after periods of significant rainfall on August 9 (19.4 mm) and August 21 (89.5 mm). On September 5, after the onset of leaf senescence, there was also a small increase in A' under elevated [CO_2].

Seasonal patterns of c_i , c_i/c_a and E

Under elevated [CO_2], c_i was greater than under ambient [CO_2] (Table 2; $F_{1,4} = 16.96$, $P = 0.006$). On July 11 low g_s and high A resulted in unreliable estimates of

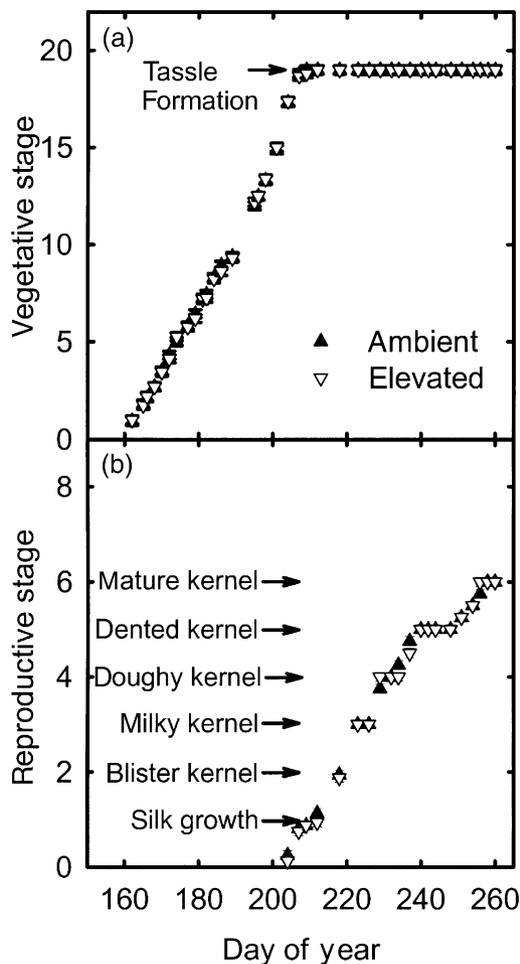


Fig. 5 Progression of *Zea mays* (a) vegetative and (b) reproductive crop ontogenetic development under ambient ($370 \mu\text{mol mol}^{-1}$; \blacktriangle) and elevated [CO_2] ($550 \mu\text{mol mol}^{-1}$; ∇) during 2002 at SoyFACE, Urbana, IL. Each stage of vegetative development corresponds to the formation of a leaf node (Ritchie *et al.*, 1993). Each point is the mean (\pm SE) of the replicate plots measured at that time ($n = 4$).

c_i . With this possible exception, c_i under elevated [CO_2] was never significantly below $200 \mu\text{mol mol}^{-1}$. By contrast, under ambient [CO_2] c_i only exceeded $200 \mu\text{mol mol}^{-1}$ on August 21. However, there were no significant differences in c_i/c_a between ambient and elevated [CO_2] (Table 2; $F_{1,4} = 0.55$, $P = 0.486$). On average across the season, E was lower under elevated [CO_2] than ambient [CO_2] by 18% (Table 2; $F_{1,4} = 13.43$, $P = 0.002$).

Rainfall from 1889 to 2001 and rainfall in 2002

Total summer rainfall (June, July and August) at SoyFACE in 2002 was 321 mm (Fig. 7a). This was almost identical to the 50-year running average of total

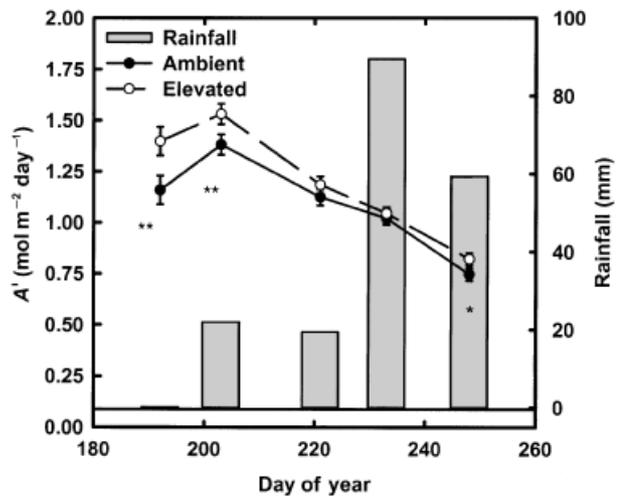


Fig. 6 Diurnal photosynthetic carbon gain (A' ; $\text{mol m}^{-2} \text{day}^{-1}$) of the youngest fully expanded leaf of *Zea mays* grown under ambient ($370 \mu\text{mol mol}^{-1}$; \bullet) and elevated [CO_2] ($550 \mu\text{mol mol}^{-1}$; \circ) on five dates during 2002 at SoyFACE, Urbana, IL. Each point is the mean (\pm SE) of the replicate plots measured at that time ($n = 4$). Statistically significant differences between treatments within measurement periods are indicated by asterisks: * $P < 0.05$, ** $P < 0.01$. Total rainfall (mm) at the field site during the prior 2 weeks is presented for each day of gas exchange measurements.

summer rainfall at nearby Urbana, IL for 2002. It was also greater than the average across all available records for Urbana (285 mm; 1889–2002). The 50-year running average of total summer rainfall increased considerably from 255 mm in 1938 to 312 mm in 2002.

Meanwhile, variability in summer rainfall (expressed as the CV between total rainfall in the summer months) in 2002 was considerably lower than the 50-year running average of monthly rainfall at Urbana (Fig. 7b). The variability of summer rainfall also increased from 1938 to 2002, as the 50-year running average CV of monthly rainfall increased by 11%.

Discussion

Counter to our prediction, there was a marked and significant increase in leaf photosynthesis of this field crop of *Z. mays* grown under continuous open-air elevation of [CO_2] to the level predicted for 2050. There was, however, significant variation in the response with date, from a 21% increase in A' on July 11 to no increase on August 9. On average, growth at elevated CO_2 resulted in 10% greater A' across the season. It is difficult to compare these results to previous studies, since this is the first to examine *Z. mays* under open-air enrichment with normal atmospheric coupling. Further,

Table 2 Intercellular CO₂ concentration (c_i ; $\mu\text{mol mol}^{-1}$), ratio of intercellular to atmospheric CO₂ concentrations (c_i/c_a) and transpiration (E ; $\text{mmol m}^{-2}\text{s}^{-1}$) of the youngest fully expanded leaf of *Zea mays* grown under ambient (370 $\mu\text{mol mol}^{-1}$) and elevated [CO₂] (550 $\mu\text{mol mol}^{-1}$) at midday on 5 days of the year (DOY) in 2002 at SoyFACE, Urbana, IL

DOY	c_i ($\mu\text{mol mol}^{-1}$)			c_i/c_a			E ($\text{mmol m}^{-2}\text{s}^{-1}$)		
	Ambient	Elevated	P	Ambient	Elevated	P	Ambient	Elevated	P
192	–	–	–	–	–	–	3.2 ± 0.4	3.4 ± 0.4	ns
203	146 ± 5	248 ± 5	***	0.40 ± 0.06	0.46 ± 0.06	ns	5.3 ± 0.1	4.8 ± 0.1	***
221	108 ± 48	191 ± 48	ns	0.29 ± 0.06	0.34 ± 0.06	ns	3.8 ± 0.4	3.4 ± 0.4	ns
233	214 ± 30	282 ± 30	*	0.58 ± 0.06	0.52 ± 0.06	ns	6.3 ± 0.4	4.9 ± 0.4	**
248	132 ± 15	232 ± 15	***	0.36 ± 0.06	0.42 ± 0.06	ns	3.4 ± 0.1	2.8 ± 0.1	***

Estimates of c_i on DOY 192 were unreliable and therefore are not presented.

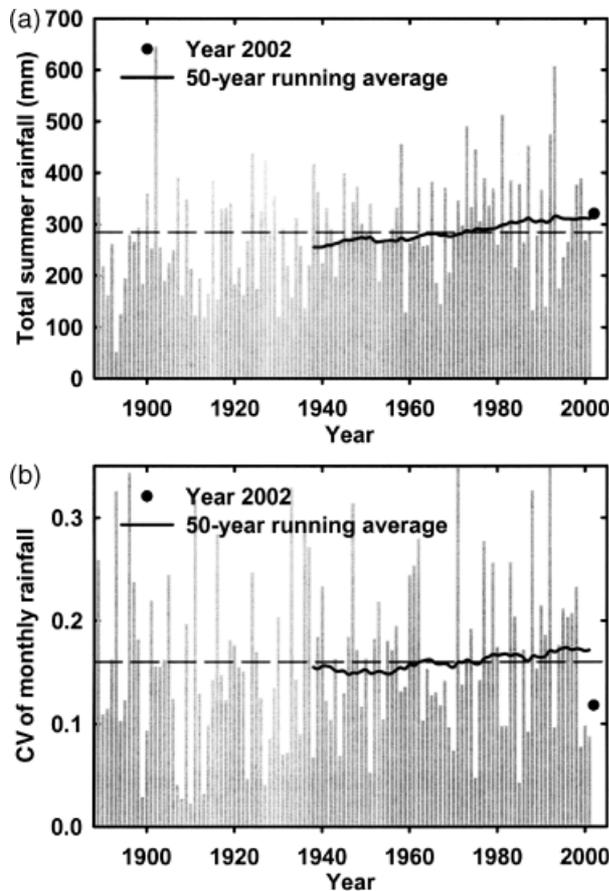


Fig. 7 (a) A histogram of the total summer rainfall (mm) in June, July and August; and (b) a histogram of the coefficient of variation (CV) between the monthly rainfall in June, July and August each year from 1889 to 2002 at the Urbana weather station (data from Illinois State Meteorological Service). On each graph the mean for the total measurement period (dashed line) and the 50-year running mean (solid line) are compared with the total rainfall and CV of rainfall measured at SoyFACE, Urbana, IL for June, July and August 2002 (closed circle).

most prior studies have examined light-saturated photosynthesis at one point in the day and one or two points in the growth cycle (e.g. Ziska & Bunce, 1997; LeCain & Morgan, 1998). These results concern the course of photosynthesis from dawn–dusk and time points from vegetative growth to late grain filling. However, it is clear that despite the mesic growing conditions, even the moderate elevation of [CO₂] to anticipated 2050 levels results in a significant increase in net CO₂ uptake.

The variability of published data on the photosynthetic responses to elevated [CO₂] of *Z. mays* makes prediction of future crop productivity extremely tentative. For example, in a comparative chamber study of 10 C₄ species, *Z. mays* showed the smallest enhancement of A under double-ambient [CO₂] (5%; Ziska & Bunce, 1997). In contrast, Kang *et al.* (2002) reported enhancements of A under double-ambient [CO₂] (700 $\mu\text{mol mol}^{-1}$) by 15%, 19% and 47% in high, medium and low water availability treatments, respectively. At the FACE experiment in the desert conditions at Maricopa, AZ, A in *Sorghum bicolor* at midday was stimulated under elevated [CO₂] (550 $\mu\text{mol mol}^{-1}$) by 23% under ‘limited irrigation’ and 9% under ‘ample irrigation’ (Wall *et al.*, 2001). This is comparable with the 10% enhancement of A' across the season in the present experiment.

Improved plant water status is considered to be the primary basis of greater A under elevated [CO₂] in C₄ plants (Seneweera *et al.*, 1998; Ghannoum *et al.*, 2000). However, this response to elevated [CO₂] would seemingly lead to enhanced A only if plants are water-stressed under ambient [CO₂]. The diurnal courses of gas exchange measured over the growing season at SoyFACE were consistent with the episodic occurrence of this scenario. The enhancement of A under elevated [CO₂] was strongest (+41%) on July 11 after a period of low rainfall. It coincided with the period of high PFD, D and T_{air} between midmorning

and late afternoon (Figs 2 and 3). Leaf curling is an indicator of leaf water stress in maize. Leaf curling was frequently observed to coincide with periods of high evaporative demand in plants grown at ambient [CO₂], but not those grown at elevated [CO₂]. After 21.9 mm of rainfall, rates of *A* under elevated [CO₂] on July 22 were still high. However, the enhancement effect of elevated [CO₂] had declined, as rates of *A* under ambient [CO₂] recovered. On August 9, after further rainfall, the [CO₂] effect was only weakly evident during a midday depression of *A* in ambient grown plants. On August 21 and September 5, after high rainfall, *A* was not significantly greater under elevated [CO₂], at any time of day. At this time no leaf curling occurred. The progressive increase in *A* observed under ambient [CO₂], relative to elevated [CO₂], is consistent with a recovery from inhibition due to drought stress as water availability increased.

Low soil water availability and high evaporative demand can both generate water stress and inhibit *A* in C₄ plants (Passioura, 1988; Grantz, 1990; Samarakoon & Gifford, 1995; Salah & Tardieu, 1997; Seneweera *et al.*, 1998). The lower *g*_s and *E* observed here under elevated [CO₂] have previously been demonstrated to: (a) reduce soil water extraction, delaying onset or reducing intensity of drought during low rainfall (Owensby *et al.*, 1997; Ghannoum *et al.*, 2000); and (b) reduce shoot water stress during periods of high evaporative demand (Salah & Tardieu, 1997; Ghannoum *et al.*, 2000). In the present study these mechanisms may have counteracted the development of water stress under elevated [CO₂] and prevented the inhibition of *A* observed under ambient [CO₂].

C₄ grasses in controlled-environment studies have displayed acclimation to elevated [CO₂] through lower carboxylation efficiency and C₄-cycle enzyme activities (Maroco *et al.* 1999; Watling *et al.*, 2000). Data were not collected to specifically investigate this phenomenon in the present study. However, the CO₂ effect on *A* was variable over the season, despite consistently greater *c*_i at elevated [CO₂]. If acclimation did occur it was insufficient to prevent a significant stimulation of *A*' across the season.

Mean canopy temperatures at midday on the days of gas-exchange measurements were, on average, ~1 °C greater in the elevated [CO₂] treatment, most likely as a result of lower *g*_s (Bernacchi *et al.* unpublished data). However, Crafts-Brandner & Salvucci (2002) suggests that leaf temperatures were within the range, close to the temperature optimum of photosynthesis in maize, where the effect upon *A* would have been negligible.

Was the greater *A* under elevated [CO₂] in 2002 a result of atypical water stress conditions? In order to address this question, rainfall patterns at SoyFACE in

2002 were compared with the meteorological record (1889–2002) of nearby Urbana. Total rainfall during the summer growing season (June–August) in 2002 (321 mm) was slightly greater than the 50-year running average (311 mm). This, together with a substantially lower than average variation in rainfall between months (CV = 0.12 in 2002 vs. a mean CV = 0.17 between 1951 and 2001), suggests that the crop did not experience atypical water stress during 2002. Nonetheless, and contrary to expectations, this US Corn Belt summer climate appeared to cause sufficient water stress under ambient [CO₂] to allow the ameliorating effects of elevated [CO₂] to significantly enhance *A*.

Current models predict only small changes in the total precipitation of central North America over this century, with the greater probability of increases than decreases by 2100 (Georgi *et al.*, 2001). However, variation in monthly precipitation and the occurrence of droughts are both projected to increase significantly in central North America (Gregory *et al.*, 1997; Beersma & Buishand, 1999). If so, future crops will experience more significant water stress than in 2002, alongside elevated [CO₂]. The typical magnitude of photosynthetic enhancement in *Z. mays* under elevated [CO₂] may, therefore, be greater than the average of 10% observed during 2002. The enhancement of *A*' by 21% on July 11, 2002 serves as an upper limit for potential enhancement at this time. This response of *Z. mays* to elevated [CO₂] indicates the potential for greater future crop biomass and harvestable yield across the US Corn Belt.

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References

- Alexandrov VA, Hoogenboom G (2000) Vulnerability and adaptation assessments of agricultural crops under climate change in the Southeastern USA. *Theoretical and Applied Climatology*, **67**, 45–63.
- Amthor JS, Mitchell RJ, Runion GB *et al.* (1994) Energy content, construction cost and phytomass accumulation of *Glycine max* (L.) Merr and *Sorghum bicolor* (L.) Moench grown in elevated CO₂ in the field. *New Phytologist*, **128**, 443–450.

- Anderson LJ, Maherali H, Johnson HB *et al.* (2001) Gas exchange and photosynthetic acclimation over subambient to elevated CO₂ in a C₃–C₄ grassland. *Global Change Biology*, **7**, 693–707.
- Beersma JJ, Buishand TA (1999) A simple test for equality of variances in monthly climate data. *Journal of Climate*, **12**, 1770–1779.
- Brown RA, Rosenberg NJ (1999) Climate change impacts on the potential productivity of corn and winter wheat in their primary United States growing regions. *Climatic Change*, **41**, 73–107.
- Crafts-Brandner SJ, Salvucci ME (2002) Sensitivity of photosynthesis in a C₄ plant, maize, to heat stress. *Plant Physiology*, **129**, 1773–1780.
- Dai ZY, Ku MSB, Edwards GE (1995) C₄ photosynthesis – the effects of leaf development on the CO₂-concentrating mechanism and photorespiration in maize. *Plant Physiology*, **107**, 815–825.
- Furbank RT, Jenkins CLD, Hatch MD (1989) CO₂ concentrating mechanism of C₄ photosynthesis – permeability of isolated bundle sheath-cells to inorganic carbon. *Plant Physiology*, **91**, 1364–1371.
- Georgi F, Hewitson B, Christensen J *et al.* (2001) Regional climate information – evaluation and predictions. In: *Climate Change 2001: The Scientific Basis. Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change* (eds Houghton JT, Ding Y, Griggs DJ), Cambridge University Press, New York, USA.
- Ghannoum O, Von Caemmerer S, Ziska LH *et al.* (2000) The growth response of C₄ plants to rising atmospheric CO₂ partial pressure: a reassessment. *Plant Cell and Environment*, **23**, 931–942.
- Grant DA (1990) Plant response to atmospheric humidity. *Plant, Cell and Environment*, **13**, 667–679.
- Gregory JM, Mitchell JFB, Brady AJ (1997) Summer drought in northern midlatitudes in a time-dependent CO₂ climate experiment. *Journal of Climate*, **10**, 662–686.
- Hendrey GR, Lewin KF, Nagy J (1993) Free air carbon-dioxide enrichment – development, progress, results. *Vegetatio*, **104**, 17–31.
- Hocking PJ, Meyer CP (1991) Effects of CO₂ enrichment and nitrogen stress on growth, and partitioning of dry-matter and nitrogen in wheat and maize. *Australian Journal of Plant Physiology*, **18**, 339–356.
- Houghton JT, Ding Y, Griggs DJ *et al.*, eds (2001) *Climate Change 2001: The Scientific Basis. Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, New York, USA.
- Jenkins CLD, Furbank RT, Hatch MD (1989) Mechanism of C₄ photosynthesis – a model describing the inorganic carbon pool in bundle sheath-cells. *Plant Physiology*, **91**, 1372–1381.
- Kang SZ, Zhang FC, Hu XT *et al.* (2002) Benefits of CO₂ enrichment on crop plants are modified by soil water status. *Plant and Soil*, **238**, 69–77.
- Knapp AK, Hamerlynck EP, Owensby CE (1993) Photosynthetic and water relations responses to elevated CO₂ in the C₄ grass *Andropogon gerardii*. *International Journal of Plant Sciences*, **154**, 459–466.
- LeCain DR, Morgan JA (1998) Growth, gas exchange, leaf nitrogen and carbohydrate concentrations in NAD-ME and NADP-ME C₄ grasses grown in elevated CO₂. *Physiologia Plantarum*, **102**, 297–306.
- McLeod A, Long SP (1999) FACE in global change research: a review. *Advances in Ecological Research*, **28**, 1–55.
- Maroco JP, Edwards GE, Ku MSB (1999) Photosynthetic acclimation of maize to growth under elevated levels of carbon dioxide. *Planta*, **210**, 115–125.
- Miglietta F, Peressotti A, Vaccari FP *et al.* (2001) Free-air CO₂ enrichment (FACE) of a poplar plantation: the POPFACE fumigation system. *New Phytologist*, **150**, 465–476.
- Moore BD, Cheng SH, Edwards GE (1986) The influence of leaf development on the expression of C₄ metabolism in *Flaveria trinervia*, a C₄ dicot. *Plant and Cell Physiology*, **27**, 1159–1167.
- Owensby CE, Ham JM, Knapp AK *et al.* (1997) Water vapour fluxes and their impact under elevated CO₂ in a C₄-tallgrass prairie. *Global Change Biology*, **3**, 189–195.
- Passioura JB (1988) Root signals control leaf expansion in wheat seedlings growing in drying soil. *Australian Journal of Plant Physiology*, **15**, 687–693.
- Poorter H, Roumet C, Campbell BD (1996) Interspecific variation in the growth response of plants to elevated CO₂: a search for functional types. In: *Carbon Dioxide, Populations and Communities* (eds Korner C, Bazzaz FA), Academic Press, New York.
- Ritchie SW, Hanway JJ, Benson GO (1993) *How a corn plant develops*. Special report number 48, Iowa State University of Science and Technology.
- Rosegrant MW, Paisner MS, Meijer S *et al.* (2001) *Global food projections to 2020: emerging trends and alternative futures*. International Food Policy Research Institute.
- Sage RF, Kubien DS (2003) *Quo vadis C₄?* An ecophysiological perspective on global change and the future of C₄ plants. *Photosynthesis Research*, in press.
- Salah HBH, Tardieu F (1997) Control of leaf expansion rate of droughted maize plants under fluctuating evaporative demand – A superposition of hydraulic and chemical messages? *Plant Physiology*, **114**, 893–900.
- Saliendra NZ, Meinzer FC, Perry M *et al.* (1996) Associations between partitioning of carboxylase activity and bundle sheath leakiness to CO₂, carbon isotope discrimination, photosynthesis, and growth in sugarcane. *Journal of Experimental Botany*, **47**, 907–914.
- Samarakoon AB, Gifford RM (1995) Soil water content under plants at high CO₂ concentration and interactions with the direct CO₂ effects: a species comparison. *Journal of Biogeography*, **22**, 193–202.
- Samarakoon AB, Gifford RM (1996) Elevated CO₂ effects on water use and growth of maize in wet and drying soil. *Australian Journal of Plant Physiology*, **23**, 53–62.
- Seneweera SP, Ghannoum O, Conroy J (1998) High vapour pressure deficit and low soil water availability enhance shoot growth responses of a C₄ grass (*Panicum coloratum* cv. Bambatsi) to CO₂ enrichment. *Australian Journal of Plant Physiology*, **25**, 287–292.
- Steel RGD, Torrie JH, Dickey DA (1997) *Principles and procedures of statistics: a biometrical approach*. McGraw-Hill, NY, USA.

- von Caemmerer S, Farquhar GD (1981) Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. *Planta*, **153**, 376–387.
- Wall GW, Brooks TJ, Adam R *et al.* (2001) Elevated atmospheric CO₂ improved Sorghum plant water status by ameliorating the adverse effects of drought. *New Phytologist*, **152**, 231–248.
- Ward SJE, Midgley GF, Jones MH *et al.* (1999) Responses of wild C₄ and C₃ grass (Poaceae) species to elevated atmospheric CO₂ concentration: a meta-analytic test of current theories and perceptions. *Global Change Biology*, **5**, 723–741.
- Wang J, Erda L (1996) The impact of potential climate change and climate variability on simulated maize production in China. *Water, Air and Soil Pollution*, **92**, 75–85.
- Watling JR, Press MC (1997) How is the relationship between the C₄ cereal *Sorghum bicolor* and the C₃ root hemi-parasites *Striga hermonthica* and *Striga asiatica* affected by elevated CO₂? *Plant Cell and Environment*, **20**, 1292–1300.
- Watling JR, Press MC, Quick WP (2000) Elevated CO₂ induces biochemical and ultrastructural changes in leaves of the C₄ cereal sorghum. *Plant Physiology*, **123**, 1143–1152.
- Wong SC (1979) Elevated atmospheric partial pressure of CO₂ and plant growth. I. Interactions of nitrogen nutrition and photosynthetic capacity in C₃ and C₄ plants. *Oecologia*, **44**, 68–74.
- Young KJ, Long SP (2000) Crop ecosystem responses to climatic change: maize and sorghum. In: *Climate change and global crop productivity* (eds Reddy KR, Hodges HF), pp. 107–131. CABI International, Oxon, UK.
- Ziska LH, Bunce JA (1997) Influence of increasing carbon dioxide concentration on the photosynthetic and growth stimulation of selected C₄ crops and weeds. *Photosynthesis Research*, **54**, 199–208.
- Ziska LH, Hogan KP, Smith AP *et al.* (1991) Growth and photosynthetic response of 9 tropical species with long-term exposure to elevated carbon-dioxide. *Oecologia*, **86**, 383–389.
- Ziska LH, Sicher RC, Bunce JA (1999) The impact of elevated carbon dioxide on the growth and gas exchange of three C₄ species differing in CO₂ leak rates. *Physiologia Plantarum*, **105**, 74–80.