# Hourly and seasonal variation in photosynthesis and stomatal conductance of soybean grown at future CO$_2$ and ozone concentrations for 3 years under fully open-air field conditions

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## ABSTRACT

It is anticipated that enrichment of the atmosphere with CO$_2$ will increase photosynthetic carbon assimilation in C3 plants. Analysis of controlled environment studies conducted to date indicates that plant growth at concentrations of carbon dioxide ([CO$_2$]) anticipated for 2050 (~ 550 $\mu$mol mol$^{-1}$) will stimulate leaf photosynthetic carbon assimilation ($A$) by 20 to 40%. Simultaneously, concentrations of tropospheric ozone ([O$_3$]) are expected to increase by 2050, and growth in controlled environments at elevated [O$_3$] significantly reduces $A$. However, the simultaneous effects of both increases on a major crop under open-air conditions have never been tested. Over three consecutive growing seasons > 4700 individual measurements of $A$, photosynthetic electron transport ($J_{PSII}$) and stomatal conductance ($g_s$) were measured on *Glycine max* (L.) Merr. (soybean). Experimental treatments used free-air gas concentration enrichment (FACE) technology in a fully replicated, factorial complete block design. The mean $A$ in the control plots was 14.5 $\mu$mol m$^{-2}$ s$^{-1}$. At elevated [CO$_2$], mean $A$ was 24% higher and the treatment effect was statistically significant on 80% of days. There was a strong positive correlation between daytime maximum temperatures and mean daily integrated $A$ at elevated [CO$_2$], which accounted for much of the variation in CO$_2$ effect among days. The effect of elevated [CO$_2$] on photosynthesis also tended to be greater under water stress conditions. The elevated [O$_3$] treatment had no statistically significant effect on mean $A$, $g_s$ or $J_{PSII}$ on newly expanded leaves. Combined elevation of [CO$_2$] and [O$_3$] resulted in a slightly smaller increase in average $A$ than when [CO$_2$] alone was elevated, and was significantly greater than the control on 67% of days. Thus, the change in atmospheric composition predicted for the middle of this century will, based on the results of a 3 year open-air field experiment, have smaller effects on photosynthesis, $g_s$ and whole chain electron transport through photosystem II than predicted by the substantial literature on relevant controlled environment studies on soybean and likely most other C3 plants.

*Key-words*: air pollution; chlorophyll fluorescence; climate change; free-air gas concentration enrichment (FACE); global change.

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## INTRODUCTION

The response of soybean [*Glycine max* (L.) Merr.] to anthropogenically driven perturbations in atmospheric trace gases has been widely studied in recognition of its potential for economic impact. Two meta-analyses have summarized the responses of soybean to growth in elevated atmospheric concentrations of CO$_2$ ([CO$_2$]; Ainsworth *et al.* 2002), elevated tropospheric ozone concentrations ([O$_3$]) and a combination of the two gases in enclosed environments (Morgan, Ainsworth & Long 2003). Leaf-level gas exchange measurements show soybean photosynthetic carbon assimilation ($A$) generally increases with growth in elevated [CO$_2$] (Ainsworth *et al.* 2002) and decreases when grown in elevated [O$_3$] (Morgan *et al.* 2003). Lower stomatal conductance ($g_s$) is associated with growth under higher concentrations of both gases (Ainsworth *et al.* 2002; Morgan *et al.* 2003). Despite these generalized responses, there are no studies that have explored full diurnal and

seasonal responses of soybean, or any other plant, to growth in elevated $[O_3]$ over the full life cycle and under fully open-air conditions. Such data have only been provided for elevated $[CO_2]$ previously for wheat (Garcia *et al.* 1998) and soybean (Rogers *et al.* 2004), and in each case only for a single year.

Evidence clearly demonstrates different directional responses of instantaneous leaf-level carbon assimilation ($A$) for soybean grown in elevated $[CO_2]$ versus $[O_3]$ (e.g. Ainsworth *et al.* 2002; Morgan *et al.* 2003). The mechanisms driving the responses in $A$ differ substantially between $[CO_2]$ and $[O_3]$. Growth in elevated $[CO_2]$ stimulates $A$ through an increase in the primary substrate, $CO_2$, and a decrease in photorespiration. As a result of this, a 37% increase in light-saturated steady-state photosynthesis and 11% increase in light-limited photosynthesis are predicted when $[CO_2]$ is increased from 370 to 550 $\mu$mol mol$^{-1}$ at 25 °C (Farquhar, Caemmerer & Berry 1980; Long 1991; Long *et al.* 2004; Rogers *et al.* 2004). This predicts that stimulation of $A$ at elevated $[CO_2]$ should be greatest during sunny weather and in the middle of the day, and least around dawn and dusk. Enhancement of $A$ by elevated $[CO_2]$ in soybean grown in enclosures has often equalled theoretical expectations (e.g. Ainsworth *et al.* 2002). However, smaller than predicted stimulation was observed for soybean, and other crops, grown in elevated $[CO_2]$ under field conditions using FACE technology (Garcia *et al.* 1998; Ainsworth *et al.* 2003; Rogers *et al.* 2004; Bernacchi *et al.* 2005). Increases in $A$ with elevated $[CO_2]$ may be diminished by numerous factors including (1) decreased maximum rate of carboxylation at ribulose-1, 5-bisphosphate carboxylase/oxygenase (RUBISCO) ($V_{c,max}$); (2) decreased maximum whole chain rate of photosynthetic electron transport ($J_{max}$), which is proportional to the rate of regeneration of RuBP (Xu, Gifford & Chow 1994; Vu *et al.* 1997; Luo, Sims & Griffin 1998; Ainsworth *et al.* 2002; Ainsworth *et al.* 2004); and (3) decreased $g_s$ (Lawlor & Mitchell 1991; Allen *et al.* 1998; Ainsworth *et al.* 2002; Polley 2002; Rogers *et al.* 2004; Bernacchi *et al.* 2005). In the field, with a continually fluctuating environment, photosynthesis will rarely achieve a true steady state (e.g. Leakey *et al.* 2002).

Much less is known regarding the mechanism of response to growth in elevated $[O_3]$, although a decrease in $A$ is typically observed via damage to photosynthetic machinery; caused primarily by a loss of the amount and activity of Rubisco (Pell, Eckardt & Enyedi 1992; Mckee, Farage & Long 1995; Reid, Fiscus & Burkey 1998; Long & Naidu 2002). Ozone affects plants by diffusing through the stomata into the leaf and reacting with the water of the exposed mesophyll apoplast and its solutes to form a wide range of reactive oxygen species (ROS). These in turn interact with plasma membranes and initiate a damage response. It is not possible to calculate a theoretical reduction in $A$ at a given $[O_3]$, though substantial decreases in $A$ with elevated $[O_3]$ is generally observed regardless of the enclosure type in which the treatment is applied. However, the youngest fully expanded leaves of soybean grown in elevated $[O_3]$ under field conditions do not show decreases in $A$ (Morgan *et al.* 2004). Only after prolonged exposure in full sunlight does $A$ begin to decrease with elevated $[O_3]$ (Morgan *et al.* 2004). These results were observed based on gas exchange measurements made on detached leaves measured in controlled environments. Whether this lack of response is observed over diurnal, seasonal and inter-annual time courses has yet to be determined.

True representation of responses to elevated $[CO_2]$ and $[O_3]$ should include variable conditions over short- and long-term timescales as well as over repeated complete life cycles. The influence of rising temperature (Long 1991) or water stress (Allen *et al.* 1994) on $A$ has also been predicted to interact with the effect of elevated $[CO_2]$; however, few studies have the temporal resolution to measure this interaction under field conditions. Rogers *et al.* (2004) demonstrated variability in the response of a soybean cultivar to elevated $[CO_2]$ over seven measurement dates within one growing season. A mean 25% increase in diurnal integrated leaf-level photosynthesis was observed across the growing season; however daily responses ranged from a 50% increase to no effect (Rogers *et al.* 2004). From the analysis of a single year it is not possible to know if these responses are characteristic or instead reflect weather or environmental conditions unique to that year. Rogers *et al.* (2004) did not investigate the effects of elevated $[O_3]$. All previous field studies have used open-top chambers which alter the coupling of the leaf canopy to the ozone-polluted air, which could exaggerate the effect of ozone on leaf photosynthesis and $g_s$ relative to natural coupling (Elagoz & Manning 2005).

In this field study on soybean, we test the hypotheses that (1) under fully open-air conditions, the response of photosynthesis to elevated $[CO_2]$ will be less than previously reported; (2) the responses will be affected by both plant developmental stage and time of day; (3) that the magnitude of response of elevated $[CO_2]$ on photosynthesis will vary with temperature and water stress; and (4) that no effect of elevated $[O_3]$ on photosynthesis or $g_s$ is observable in newly expanded leaves when compared with control or to elevated $[CO_2]$ treatment. In addition to measuring $A$ and $g_s$, whole chain electron transport through photosystem II ($J_{PSII}$) was determined by simultaneous modulated chlorophyll fluorescence to aid interpretation of observed responses. In total, > 4700 individual measurements each of $A$, $g_s$ and $J_{PSII}$ were made over full diurnal cycles during three complete growing seasons and life cycles. This study used the Soybean Free Air gas Concentration Enrichment (SoyFACE) facility at Champaign, IL, USA.

## METHODS

### Site description

The SoyFACE facility is contained within a 32 ha (80 acre) field of the South Farms, University of Illinois at Urbana-Champaign (40°03′21.3″N, 88°12′3.4″W, 230 m a.s.l.; www.soyface.uiuc.edu). The soil is a Drummer-Flanagan soil series (fine-silty, mixed, mesic Typic Endoaquoll)

typically very deep and formed from loess and silt parent material deposited on the glacial till and outwash plain. This site has been in continuous cultivation of arable crops for over 100 years. Soybean [*Glycine max* (L.) Merr. cv. Pioneer 93B15] and maize (*Zea mays*) each occupied one half of the field and follow an annual rotation. After the autumn maize harvest, the stubble was chopped with a mower followed by tillage with a rip chisel and conventional single-pass cultivator tillage was used in the spring. Soybean row spacing was 0.38 m and cultivation followed typical Illinois agricultural practices (Ainsworth *et al.* 2004; Rogers *et al.* 2004).

Four blocks were nested within the 16 ha of soybean, each containing one control, one elevated $[CO_2]$, one elevated $[O_3]$ and one elevated $[CO_2] + [O_3]$ treatment plot. The plots were octagonal with a mean diameter of 20 m, defined by horizontal pipes which provide the controlled release of $O_3$, $CO_2$ or both gases. These pipes and associated equipment are assembled immediately after sowing, so that treatment was applied at emergence and continued through to final harvest. The plots were separated by at least 100 m to avoid cross-contamination. Treatments were arranged in a randomized complete block design (*n* of 4 for each treatment) to control for topographic and soil variation across the field.

The target $[CO_2]$ of 550 $\mu$mol mol$^{-1}$ and $[O_3]$ of 1.23 × current concentration was based on projected future mean global tropospheric concentrations for 2050 (IPCC 2001; Prather *et al.* 2001, 2003). Seasonal average $[CO_2]$ in the elevated $[CO_2]$ and elevated $[CO_2] + [O_3]$ treatments was 552 $\mu$mol mol$^{-1}$ in 2002 and 2003, and 550 $\mu$mol mol$^{-1}$ in 2004. This compared to an ambient $[CO_2]$ of $\sim$ 375 $\mu$mol mol$^{-1}$. Seasonal average $[O_3]$ in the elevated $[O_3]$ and elevated $[CO_2] + [O_3]$ treatments was greater than ambient $[O_3]$ by 21% in 2002, and 25% in 2003 and 2004 (Fig. 1). Mean 8 h maximum $[O_3]$ throughout the season and seasonal AOT40 and SUM06 values (Mauzerall & Wang 2001) were calculated from ozone concentrations measured on site (model 49CO3 analyser; Thermo Environmental Instruments, Franklin, MA, USA; calibration US EPA Equivalent Method EQQA-0880-047, range 0 to 0.05–1.0 $\mu$mol mol$^{-1}$) from control and elevated $[O_3]$ plots (Fig. 1). Elevation of $[CO_2]$ and $[O_3]$ was based on the method of Miglietta *et al.* (2001) modified as described previously for $CO_2$ (Ainsworth *et al.* 2004) and $O_3$ (Morgan *et al.* 2004). All 3 years of measurement included the control, elevated $[CO_2]$ and elevated $[O_3]$ treatments while the elevated $[CO_2] + [O_3]$ treatment was added for 2003 and 2004.

Palmer crop moisture index (PCMI) is an estimate of short-term moisture conditions based on temperature, precipitation and modelled soil water content (Palmer 1968). As PCMI decreases below zero, it indicates progressively greater drought stress conditions. PCMI from 1973 to 2004 for East Central Illinois was provided by the Climate Operation Branch of the National Oceanic and Atmospheric Administration (NOAA) (http://www.usda.gov/oce/waob/jawf/). Meteorological measurements of precipitation,

temperature, humidity and solar radiation were collected in 15 min averages from a weather station located at SoyFACE. Thirty year mean averages (1971–2000) of meteorological variables were determined from recorded data of weather stations located within 3 km of the SoyFACE field through the Midwestern Climate Information System (MICIS) operated by the Midwestern Regional Climate Center, Illinois State Water Survey (http://mrcc.sws.uiuc.edu/).

## Gas exchange and chlorophyll fluorometry measurements

Measurements were made from predawn to post-dusk on 15 d over three complete growing seasons (Table 1). A total of three teams in 2002 and four teams in 2003 and 2004 measured leaf level gas exchange each using a portable open gas exchange systems incorporating infrared $CO_2$ and water vapor analysers (Li-Cor 6400; Li-Cor, Inc., Lincoln, NE, USA) coupled with an integrated portable chlorophyll fluorometer (LI-6400-40 leaf chamber fluorometer; Li-Cor, Inc.). Prior to measurements, the infrared $CO_2$ and water vapor analysers were zeroed using anhydrous calcium carbonate (Drierite, W.A. Hammond Drierite Company, Ltd, Xenia, OH, USA) to remove water, and NaOH/Ca(OH)$_2$ (soda lime granules, Morgan Medical, Ltd, Kent, UK) to remove $CO_2$, from the air entering the cuvettes. The gas exchange systems were calibrated every 30–45 d throughout the season using gas of a known, certified, $CO_2$ concentration with 21% oxygen and nitrogen as balance (S.J. Smith, Champaign, IL, USA), and known water vapor concentrations generated with a controlled humidification system (LI-610 Portable Dew Point Generator; Li-Cor, Inc.).

Measurements were made on the youngest fully expanded leaves from plants within a $4 \times 2$ m area in each plot reserved for this study. Measurements were initiated prior to sunrise in 2002 and 2004, and immediately after sunrise in 2003. Early morning measurements often coincided with dew present on the leaves; in which case, leaves were blotted with paper towels immediately prior to enclosure into the leaf chamber. $g_s$ was not calculated at these times, because residual surface moisture likely increased apparent water vapor fluxes from the leaf. Three leaves on separate plants in each plot were measured once approximately every 2 h. Each of the teams sampled one complete block for each time point. Each team started their cycle of measurements in a different block and cycled through the blocks over the day. This avoided confounding time within the approximately 45 min to 1 h that it took to complete all measurements with treatment and avoided confounding any differences between teams with a block.

Immediately prior to the start of a measurement cycle, the red-blue light-emitting diode (LED) light source integrated into each gas exchange system was set to the incident photosynthetically active photon flux (*I*) that was determined using a photometric sensor mounted just above the plant canopy (LI-210, Li-Cor, Inc.). Similarly, the measurement temperature of the gas exchange cuvette block was
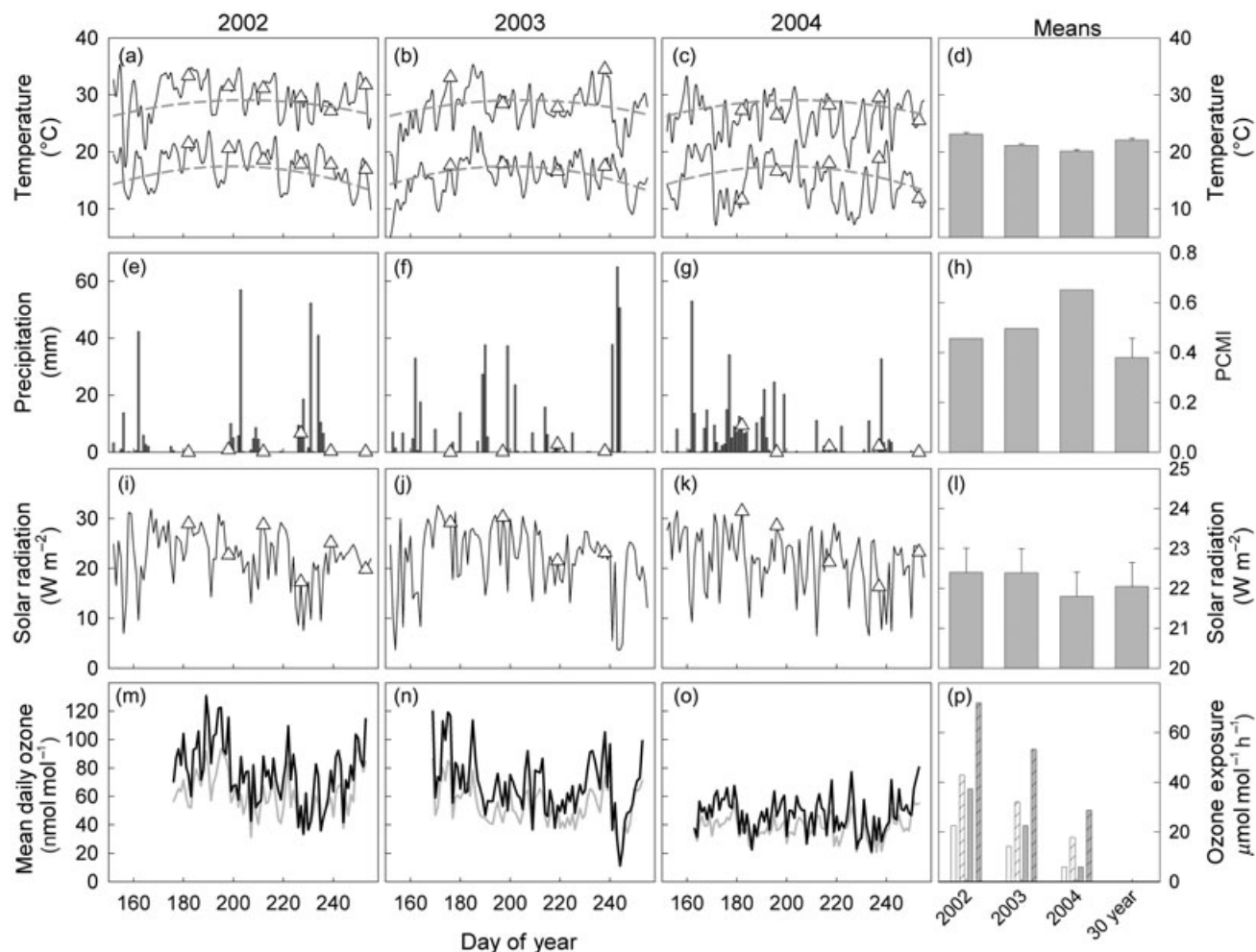
**Figure 1.** Meteorological conditions measured during the 2002, 2003 and 2004 growing seasons. Panels (a, b & c) show the daily maximum and minimum temperatures throughout each growing season (solid lines) and the daily maximum and minimum temperatures averaged for the period 1971–2000 (grey broken line). Also shown is the mean temperature for each of the three growing seasons and the 30 year mean (1971–2000, d). Daily total precipitation is shown for each of the growing seasons (e, f & g) along with mean May–September Palmer crop moisture index (PCMI) values for Illinois Climate Division 5 by the Climate Operation Branch of the National Oceanic and Atmospheric Administration (NOAA) (http://www.ncdc.noaa.gov/oa/ncdc.html; h). Total daily integral of solar radiation for each day of the growing season (i, j, k) is shown along with mean daily solar radiation for each of the growing seasons compared with the 30 year mean value. The dates on which diurnal photosynthesis ($A$), stomatal conductance ($g_s$) and whole chain electron transport through photosystem II ($J_{PSII}$) were measured are indicated by the open triangles. Mean 8 h maximum ozone concentrations are shown for each of the three growing seasons (m, n & o) along with ozone exposure calculations using the AOT40 (white bars) and SUM06 (grey bars) for the control (open bars) and elevated [O₃] (striped bars, p).

set to the open-air temperature recorded at the start of the measurement period using an aspirated shielded type T thermocouple (copper-constantan) positioned 2 m above ground and attached to a datalogger (CR7 700X Measurement and Control System; Campbell Scientific, Logan, UT, USA). Humidity in the gas exchange cuvette was not controlled except to avoid condensation inside the gas exchange system during early morning and evening measurements. The light and temperature conditions within the chamber were held constant for the duration of the measurement period, regardless of short-term fluctuations in light and air temperature. [CO₂] in the cuvettes was set to growth [CO₂] for each treatment, with the control and elevated [O₃] plots having a set point of 370 $\mu$mol mol⁻¹, and

the elevated [CO₂] and elevated [CO₂] + [O₃] plots having a set point of 550 $\mu$mol mol⁻¹. Conditions were allowed to stabilize for 45–60 s after enclosing 2 cm² of an attached leaflet in the gas exchange cuvette. The time required for one complete measurement cycle of all 16 plots ranged from 45 min to 1 h.

Calculations of $A$ and $g_s$ followed the equations of von Caemmerer & Farquhar (1981) and for $J_{PSII}$ Genty, Briantais & Baker (1989). Daily integrated rates of carbon assimilation ($A'$) and whole chain electron transport ($J'$) were calculated from the instantaneous measurements made for each day of the growing season as described previously (Rogers *et al*. 2004). In 2003 when predawn measurements were not made, the leaves were assumed to have a net

**Table 1.** Calendar date, day of year (DOY), diurnal start and finish times of experimental measurements and estimated crop ontogenetic developmental stage (defined in Ritchie *et al.* 1989) for *Glycine max* grown at Soybean Free Air Concentration Enrichment (SoyFACE) in Champaign, IL

| Date | DOY | First measurement (h) | Last measurement (h) | Growth stage |
|---|---|---|---|---|
| 1 July 2002 | 182 | 4:30 | 20:30 | Vegetative, fourth trifoliate |
| 17 July 2002 | 198 | 4:30 | 20:30 | Full bloom, flower in top 2 nodes |
| 31 July 2002 | 212 | 4:30 | 20:30 | Beginning pod in top 4 nodes |
| 15 August 2002 | 227 | 4:30 | 20:30 | 3.175 mm seed in top 4 nodes |
| 27 August 2002 | 239 | 5:00 | 20:00 | 3.175 mm seed in top 4 nodes |
| 10 September 2002 | 253 | 5:30 | 19:00 | Full size seed in top 4 nodes |
| 25 June 2003 | 176 | 7:00 | 21:00 | Vegetative, second trifoliate |
| 16 July 2003 | 197 | 7:00 | 21:00 | Beginning bloom, first flower |
| 7 August 2003 | 219 | 7:30 | 21:30 | Beginning pod in top 4 nodes |
| 26 August 2003 | 238 | 7:00 | 19:30 | 3.175 mm seed in top 4 nodes |
| 30 June 2004 | 182 | 4:30 | 20:30 | Vegetative, fourth trifoliate |
| 14 July 2004 | 196 | 4:30 | 20:30 | Beginning bloom, first flower |
| 4 August 2004 | 217 | 4:30 | 19:45 | Full pod, three-fourth inch pod in top 4 nodes |
| 24 August 2004 | 237 | 4:30 | 20:00 | 3.175 mm seed in top 4 nodes |
| 9 September 2004 | 253 | 5:00 | 19:00 | Full size seed in top 4 nodes |

carbon assimilation of zero at sunrise. A polynomial was fitted to the data and the area under the curve integrated individually for each plot throughout each day. The order of the polynomial was increased stepwise until no significant increase in $r^2$ was achieved. The resulting polynomials ranged from third to fifth order. Calculations for $A'$ and $J'$ were limited to the period of the day when $A \geq 0$; that is, net photosynthesis was positive.

## Statistical analysis

Statistical analyses were conducted using the models developed previously for SoyFACE (Rogers *et al.* 2004). The plot was identified as the sample unit, with this value being the mean of the three subsamples in a plot at a single time point. The diurnal measurements were analysed separately for each day of year (DOY) using a mixed model complete block repeated measures analysis of variance (ANOVA) (PROC MIXED, SAS v9.1, SAS Institute, Cary, NC, USA), with time of day, treatment and time of day-by-treatment as fixed effects. *A priori* determined pairwise comparisons between the treatments and the controls were performed for each measurement day. The best-fit variance/covariance matrices were chosen for each variable using Akaike's information criterion to correct for inequality of variance between the sampling time periods (Keselman *et al.* 1998; Littell, Henry & Ammerman 1998; Littell, Pendergast & Natarajan 2000). Integrated calculation of $A'$ and $J'$ was also analysed as above only with DOY, treatment and DOY-by-treatment as fixed effects. Comparison between years was conducted using a complete block ANOVA with year and treatment as main effects. Because the experiment plots were rotated to different ends of the field each year, repeated measures analysis was not appropriate. Comparisons between the percentage stimulation in photosynthesis and daily maximum temperatures or PCMI were made using a linear regression ANOVA for a first-order fit to the data (SigmaPlot, Systat Software, Inc, Richmond, CA,

USA). Given the variability associated with field studies, statistical significance is reported at $P < 0.1$ as the risk of a Type II error would otherwise be high.

## RESULTS

The measured diurnal courses were spaced throughout the growing seasons representing a range of developmental stages (Table 1), ozone concentrations and meteorological conditions (Fig. 1). Of the 3 years, 2002 had the lowest PCMI but all 3 years were higher than the 1973–2001 average (Fig. 1). Daily maximum and minimum temperatures varied substantially throughout the duration of this experiment. Mean temperature was above average in 2002, average in 2003 and below average in 2004 (Fig. 1). Total solar radiation was generally similar among the three growing seasons, with only a slight decrease in solar radiation during the 2004 growing season relative to the previous two seasons (Fig. 1). In addition to variation in temperature, measurements throughout the three growing seasons included days during dry spells (e.g. DOY 238, 2003), rain (e.g. DOY 227, 2002), clear skies (e.g. DOY 182, 2004) and overcast skies (e.g. DOY 227, 2002). Additionally, the crop experienced a hailstorm that destroyed > 50% of the plant canopy on 14 July 2003 (Morgan *et al.* 2005). Background ambient [O₃] varied within and among seasons with 2002 experiencing the highest and 2004 the lowest concentrations (Fig. 1).

Over the duration of this experiment, ca 320–430 individual leaves were measured on each of the 15 d, spread across the three growing seasons. Averaged across the daylight hours of the 15 d, mean $A$ in the control plots was 14.5 $\mu$mol m$^{-2}$ s$^{-1}$ and 24% higher in the elevated [CO₂] plots at 17.9 $\mu$mol m$^{-2}$ s$^{-1}$. Photosynthesis in the elevated [CO₂] plots was significantly greater on 80% of the measurement days. The mean $A$ for the [O₃] plots was identical to that of the controls, and plots with simultaneous elevated [CO₂] and [O₃] treatment were 19% higher than the controls and significantly higher for 67% of days (Table 2).

**Table 2.** Statistic alanalysis of the complete-block repeated measures analysis of variance for instantaneous diurnal measurements of leaf-level photosynthesis (*A*)

| Year | Day of year | Main effects | | | Pairwise comparisons | | | Means | | | |
|------|-------------|---------|---------|---------------|---------|-----|-------------|------------|------------|------------|------------|
| | | T | H | T × H | $CO_2$ | $O_3$ | $CO_2 + O_3$ | Control | $CO_2$ | $O_3$ | $CO_2 + O_3$ |
| 2002 | 182 | < 0.001 | < 0.001 | < 0.001 | < 0.001 | ns | – | 14.7 ± 0.7 | 19.3 ± 0.7 | 14.3 ± 0.7 | – |
| | 198 | < 0.01 | < 0.001 | < 0.001 | < 0.01 | ns | – | 14.7 ± 0.5 | 17.5 ± 0.5 | 14.4 ± 0.5 | – |
| | 212 | < 0.01 | < 0.001 | < 0.001 | < 0.01 | ns | – | 16.4 ± 0.9 | 20.4 ± 0.9 | 16.9 ± 0.9 | – |
| | 227 | < 0.001 | < 0.001 | < 0.001 | < 0.001 | ns | – | 13.7 ± 0.6 | 18.0 ± 0.6 | 14.1 ± 0.6 | – |
| | 239 | ns | < 0.001 | < 0.1 | ns | ns | – | 16.7 ± 0.9 | 17.7 ± 0.9 | 15.7 ± 0.9 | – |
| | 253 | < 0.1 | < 0.001 | < 0.001 | < 0.1 | ns | – | 11.2 ± 0.9 | 14.2 ± 0.9 | 9.7 ± 0.9 | – |
| 2003 | 176 | < 0.01 | < 0.001 | < 0.01 | < 0.01 | ns | < 0.01 | 14.0 ± 1.0 | 18.2 ± 1.0 | 13.8 ± 1.0 | 18.5 ± 1.0 |
| | 197 | < 0.01 | < 0.001 | < 0.1 | < 0.001 | ns | < 0.01 | 16.8 ± 1.0 | 20.5 ± 1.0 | 17.9 ± 1.0 | 20.1 ± 1.0 |
| | 219 | < 0.1 | < 0.001 | ns | ns | ns | ns | 15.5 ± 0.9 | 17.4 ± 0.9 | 15.2 ± 0.9 | 17.4 ± 0.9 |
| | 238 | < 0.1 | < 0.001 | < 0.001 | < 0.01 | ns | ns | 16.8 ± 1.3 | 22.3 ± 1.3 | 17.6 ± 1.3 | 19.7 ± 1.3 |
| 2004 | 182 | < 0.01 | < 0.001 | < 0.01 | < 0.001 | ns | < 0.01 | 15.6 ± 1.2 | 21.0 ± 1.2 | 15.9 ± 1.2 | 19.3 ± 1.2 |
| | 196 | < 0.01 | < 0.001 | < 0.1 | < 0.1 | ns | < 0.1 | 13.7 ± 0.6 | 15.8 ± 0.6 | 12.8 ± 0.6 | 15.0 ± 0.6 |
| | 217 | < 0.1 | < 0.001 | ns | ns | < 0.1 | ns | 14.7 ± 0.8 | 15.0 ± 0.8 | 13.4 ± 0.8 | 15.3 ± 0.8 |
| | 237 | < 0.01 | < 0.001 | < 0.001 | < 0.001 | ns | < 0.01 | 13.3 ± 0.8 | 16.9 ± 0.8 | 13.7 ± 0.8 | 16.3 ± 0.8 |
| | 253 | < 0.1 | < 0.001 | ns | < 0.1 | ns | < 0.1 | 8.8 ± 1.4 | 14.0 ± 1.4 | 11.9 ± 1.4 | 13.8 ± 1.4 |

The main effects are treatment (T), hour (H) and T × H, with H as a repeated term. Pairwise comparisons of the three treatments versus control were determined *a priori*. Significance of *F* values is given in the table where non-statistical significance (ns) > 0.1. Also shown are mean daily values of *A* for each treatment on each date (± one SE of the mean).

$J_{PSII}$ averaged across all days and times averaged 132 $\mu$mol $m^{-2}$ $s^{-1}$ in the control plots and was 9% higher at 143 $\mu$mol $m^{-2}$ $s^{-1}$ in the elevated $[CO_2]$ treatment. $J_{PSII}$ was also greater in the elevated $[CO_2] + [O_3]$ treatment than the control, but only by 3%, while there was no effect of $[O_3]$ alone. Given the many measurements, only representative figures showing the diurnal course of *A* and $J_{PSII}$ from each growing seasons are included (Fig. 2; all data collected throughout this experiment may be viewed in the online supplementary information). Over all three growing seasons, elevated $[CO_2]$ and elevated $[CO_2] + [O_3]$ grown leaves show higher *A*, but the stimulation was only apparent in the middle of the day (Fig. 2). This accounts for the significant treatment by time effect seen most days (Table 2). The pattern of response observed for $J_{PSII}$ was similar to *A*, with elevated $[CO_2]$ grown plants showing higher rates than the control (Fig. 2). However the proportional increase, relative to control was only about half that observed for *A* and only significant on 40% of days (Table 3; Fig. 2). When plants were grown in elevated $[O_3]$ and $[CO_2]$, a significant increase in $J_{PSII}$ was only apparent on 33% of days, and when grown in elevated $[O_3]$ alone a significant decrease in $J_{PSII}$ was observed on 1 d (Table 3). Two of the measurement dates in which a statistically significant mean effect of elevated $[CO_2]$ was lacking showed a significant time by treatment response. Further investigation of these days shows certain time periods throughout the day when $J_{PSII}$ is statistically higher for elevated $[CO_2]$ than control grown plants.

Representative plots of diurnal $g_s$ are shown for each of the 3 years (Fig. 3). Averaged across the 15 d, the mean $g_s$ in the control plots was 0.46 mol $m^{-2}$ $s^{-1}$ and 16% lower in the elevated $[CO_2]$ plots at 0.38 mol $m^{-2}$ $s^{-1}$. Decreases in $g_s$ were statistically significant on 53% of measurement days as a result of growth in elevated $[CO_2]$ (Table 4). The average $g_s$ for plants grown under elevated $[O_3]$ was only 1% lower than of the controls and not significantly different, except on 2 d in 2003. With the exception of 1 d the elevated $[CO_2] + [O_3]$ grown plants mirrored the responses of the elevated $[CO_2]$ grown plants (Table 4).

Daily integral of *A* (*A*′) was typically higher for the plants grown at elevated $[CO_2]$ and elevated $[CO_2] + [O_3]$ over the duration of the experiment (Fig. 4). Increases in seasonal *A*′ ranged from 18% in 2004 to 25% in 2002 for the elevated $[CO_2]$ grown plants and from 16% in 2004 to 20.5% in 2003 for the elevated $[CO_2] + [O_3]$ grown plants (Table 5). There was a significant treatment-by-day interaction in 2002 and 2004, suggesting a variable treatment response over the duration of these growing seasons (Fig. 4; Table 5).

A comparison of *A*′ between control and elevated $[CO_2]$ grown plants for the 3 years showed differences among years and treatments ($P < 0.001$) but no significant interaction. Analysing this further with pairwise comparisons showed that there was no difference between 2002 and 2003 but *A*′ was significantly lower in 2004 (Fig. 4). Per cent stimulation of *A*′ by elevated $[CO_2]$ rose with daily maximum temperature ($r^2 = 0.51$; Fig. 5). Stimulation of *A*′ declined with increasing PCMI, which is a measure of decreasing water stress ($r^2 = 0.25$, $P = 0.0675$; Fig. 5). However, the relationship between PCMI and percentage stimulation of photosynthesis is only statistically significant when DOY 217 in 2004 is excluded from the analysis (Fig. 5).

Daily integrated rates of whole chain electron transport through photosystem II (*J*′) were also estimated from the diurnal responses (Fig. 6). The response of *J*′ to elevated $[CO_2]$ is variable, as expected from the diurnal responses;
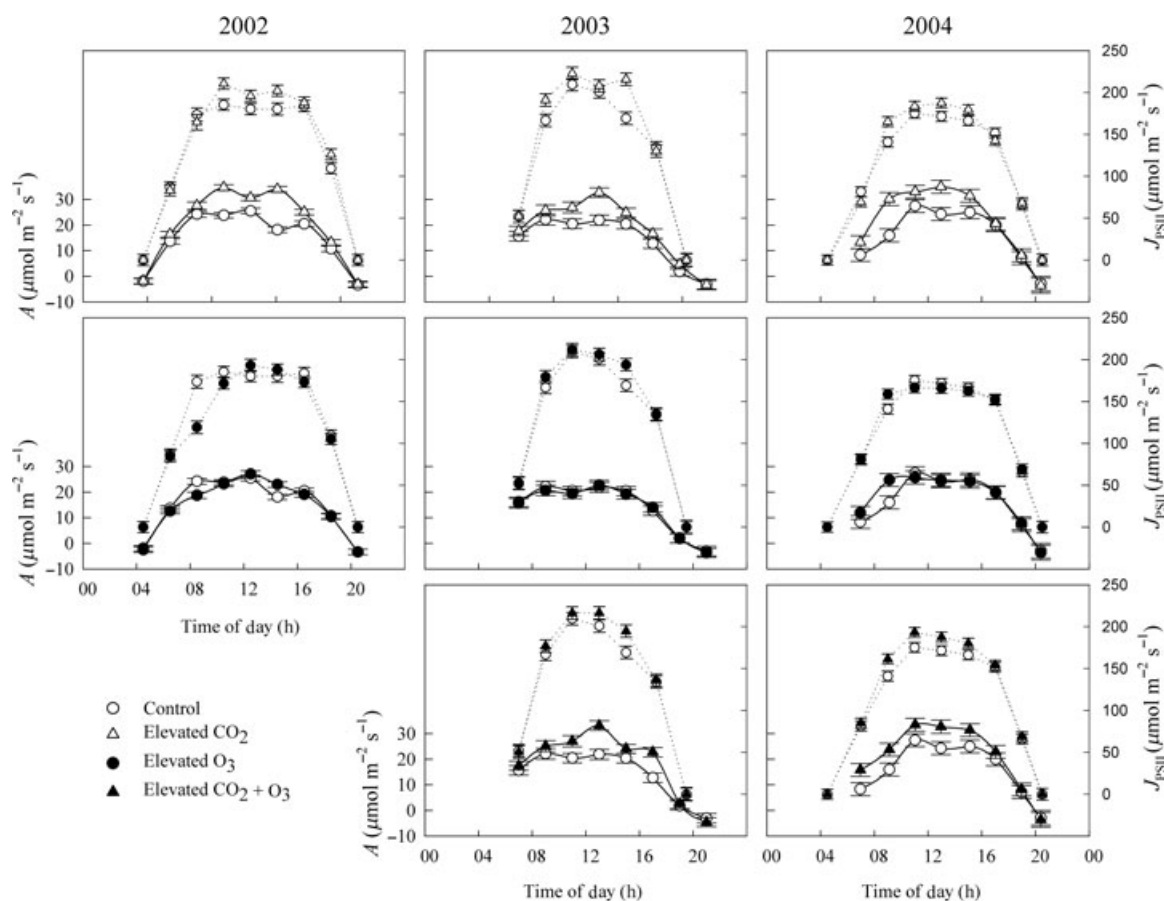
**Figure 2.** Representative measurements of $A$ (solid lines) and $J_{PSII}$ (dotted lines) over the diurnal time course from one day in 2002 (first column), 2003 (second column) and 2004 (third column). Measurements were made on day of year 182 for the 2002 and 2004 growing season, and day of year 176 during 2003. The top three panels compare control and elevated $[CO_2]$, the middle three panels control versus elevated $[O_3]$ and the bottom two panels control versus elevated $[CO_2] + [O_3]$. Open symbols represent ambient $[O_3]$, closed symbols represent elevated $[O_3]$, circles represent ambient $[CO_2]$ and triangles represent elevated $[CO_2]$. Error bars represent one SE around the mean.

**Table 3.** Statistical analysis of the complete-block repeated measures analysis of variance for instantaneous diurnal measurements of whole chain electron transport through photosystem II ($J_{PSII}$)

| | | Main effects | | | Pairwise comparisons | | | Means | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Year | Day of year | T | H | T × H | $CO_2$ | $O_3$ | $CO_2 + O_3$ | Control | $CO_2$ | $O_3$ | $CO_2 + O_3$ |
| 2002 | 182 | < 0.05 | < 0.001 | ns | < 0.05 | ns | – | 159.1 ± 3.9 | 168.3 ± 4.1 | 157.8 ± 4.2 | – |
| | 198 | ns | < 0.001 | ns | ns | ns | – | 147.6 ± 3.0 | 150.9 ± 3.0 | 147.6 ± 3.0 | – |
| | 212 | ns | < 0.001 | ns | ns | ns | – | 153.0 ± 5.1 | 164.1 ± 5.1 | 155.5 ± 5.1 | – |
| | 227 | < 0.05 | < 0.001 | ns | < 0.05 | ns | – | 127.7 ± 2.9 | 134.5 ± 2.9 | 124.8 ± 2.9 | – |
| | 239 | ns | < 0.001 | ns | ns | ns | – | 132.8 ± 4.5 | 132.0 ± 4.5 | 125.8 ± 4.5 | – |
| | 253 | < 0.05 | < 0.001 | ns | ns | < 0.05 | – | 127.6 ± 4.0 | 132.0 ± 4.0 | 115.1 ± 4.0 | – |
| 2003 | 176 | ns | < 0.001 | < 0.1 | ns | ns | ns | 167.4 ± 3.8 | 173.2 ± 3.8 | 164.6 ± 3.8 | 168.4 ± 3.8 |
| | 197 | ns | < 0.001 | ns | < 0.05 | ns | ns | 114.5 ± 4.5 | 123.2 ± 4.5 | 118.9 ± 4.5 | 119.0 ± 4.5 |
| | 219 | ns | < 0.001 | ns | ns | ns | ns | 117.1 ± 3.7 | 118.1 ± 3.7 | 118.1 ± 3.7 | 109.9 ± 3.7 |
| | 238 | < 0.1 | < 0.001 | ns | < 0.01 | ns | ns | 155.6 ± 4.3 | 170.9 ± 4.3 | 162.4 ± 4.3 | 162.6 ± 4.3 |
| 2004 | 182 | < 0.01 | < 0.001 | < 0.05 | < 0.05 | ns | < 0.001 | 136.1 ± 4.2 | 142.4 ± 4.2 | 137.4 ± 4.2 | 147.5 ± 4.2 |
| | 196 | < 0.05 | < 0.001 | ns | < 0.05 | ns | ns | 100.0 ± 3.9 | 107.6 ± 3.9 | 98.1 ± 3.9 | 103.9 ± 3.9 |
| | 217 | < 0.01 | ns | < 0.0001 | ns | ns | ns | 113.0 ± 4.0 | 108.4 ± 4.0 | 106.5 ± 4.0 | 110.9 ± 4.0 |
| | 237 | < 0.1 | < 0.001 | ns | < 0.1 | ns | < 0.1 | 125.8 ± 3.4 | 130.8 ± 3.4 | 125.0 ± 3.4 | 131.8 ± 3.5 |
| | 253 | < 0.1 | < 0.01 | ns | < 0.05 | ns | < 0.05 | 98.1 ± 4.9 | 109.2 ± 4.9 | 105.1 ± 4.9 | 109.5 ± 4.9 |

The main effects are treatment (T), hour (H) and T × H, with H as a repeated term. Pairwise comparisons of the three treatments versus control were determined *a priori*. Significance of $F$ values is given in the table where non-statistical significance (ns) > 0.1. Also shown are mean daily values of $J_{PSII}$ for each treatment on each date (± one SE of the mean).
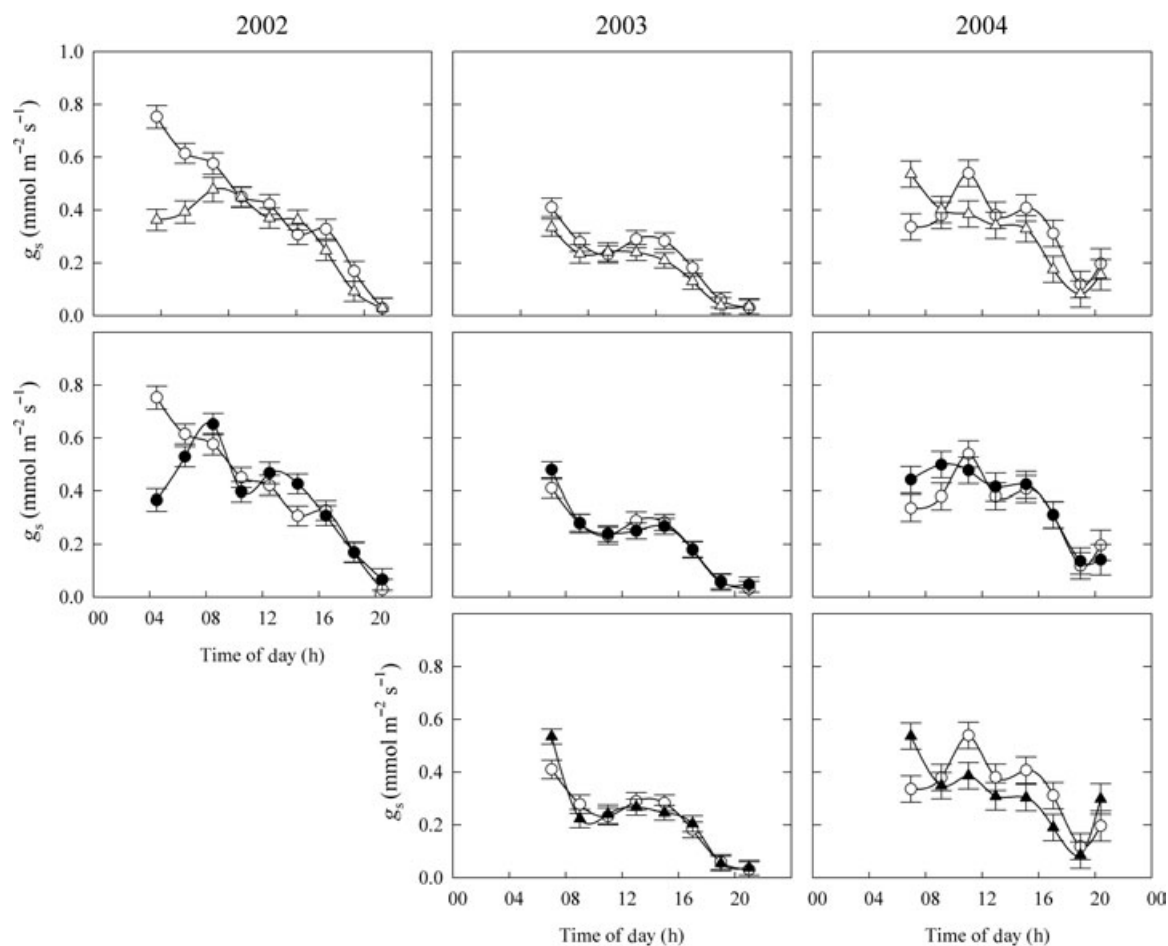
**Figure 3.** Representative measurements of stomatal conductance ($g_s$) over the diurnal time course from the days represented in Fig. 2. The top three panels compare control and elevated [$CO_2$], the middle three panels control versus elevated [$O_3$] and the bottom two panels control versus elevated [$CO_2$] + [$O_3$]. Open symbols represent ambient [$O_3$], closed symbols represent elevated [$O_3$], circles represent ambient [$CO_2$] and triangles represent elevated [$CO_2$]. Error bars represent one SE around the mean.

**Table 4.** Statistical analysis of the complete-block repeated measures analysis of variance for instantaneous diurnal measurements of stomatal conductance ($g_s$)

| Year | Day of year | Main effects | | | Pairwise comparisons | | | Means | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | T | H | T × H | $CO_2$ | $O_3$ | $CO_2$ + $O_3$ | Control | $CO_2$ | $O_3$ | $CO_2$ + $O_3$ |
| 2002 | 182 | < 0.1 | < 0.001 | ns | < 0.05 | ns | – | 0.40 ± 0.03 | 0.29 ± 0.03 | 0.38 ± 0.03 | – |
| | 198 | < 0.01 | < 0.001 | < 0.05 | < 0.001 | ns | – | 0.46 ± 0.02 | 0.33 ± 0.02 | 0.43 ± 0.02 | – |
| | 212 | ns | < 0.001 | < 0.05 | ns | ns | – | 0.57 ± 0.05 | 0.54 ± 0.05 | 0.53 ± 0.05 | – |
| | 227 | ns | < 0.001 | ns | ns | ns | – | 0.57 ± 0.04 | 0.50 ± 0.04 | 0.57 ± 0.04 | – |
| | 239 | < 0.05 | < 0.001 | ns | < 0.05 | ns | – | 0.59 ± 0.03 | 0.50 ± 0.03 | 0.62 ± 0.03 | – |
| | 253 | ns | < 0.001 | ns | ns | ns | – | 0.40 ± 0.03 | 0.40 ± 0.03 | 0.38 ± 0.03 | – |
| 2003 | 176 | < 0.05 | < 0.001 | ns | < 0.01 | ns | ns | 0.22 ± 0.01 | 0.18 ± 0.01 | 0.22 ± 0.01 | 0.22 ± 0.01 |
| | 197 | < 0.001 | < 0.001 | ns | < 0.05 | < 0.05 | < 0.05 | 0.49 ± 0.03 | 0.43 ± 0.03 | 0.55 ± 0.03 | 0.42 ± 0.03 |
| | 219 | < 0.01 | < 0.001 | ns | < 0.01 | < 0.05 | < 0.01 | 0.65 ± 0.03 | 0.48 ± 0.03 | 0.56 ± 0.03 | 0.50 ± 0.03 |
| | 238 | ns | < 0.001 | ns | ns | ns | ns | 0.36 ± 0.03 | 0.36 ± 0.03 | 0.37 ± 0.03 | 0.30 ± 0.03 |
| 2004 | 182 | ns | < 0.001 | ns | ns | ns | ns | 0.33 ± 0.03 | 0.30 ± 0.03 | 0.36 ± 0.03 | 0.31 ± 0.03 |
| | 196 | < 0.01 | < 0.001 | ns | < 0.01 | ns | < 0.01 | 0.42 ± 0.03 | 0.29 ± 0.03 | 0.41 ± 0.03 | 0.29 ± 0.03 |
| | 217 | < 0.001 | < 0.001 | < 0.05 | < 0.001 | ns | < 0.001 | 0.41 ± 0.02 | 0.25 ± 0.02 | 0.42 ± 0.02 | 0.30 ± 0.02 |
| | 237 | ns | < 0.1 | ns | ns | ns | ns | 0.63 ± 0.08 | 0.57 ± 0.08 | 0.66 ± 0.08 | 0.58 ± 0.08 |
| | 253 | ns | < 0.001 | ns | ns | ns | ns | 0.38 ± 0.05 | 0.34 ± 0.04 | 0.31 ± 0.04 | 0.40 ± 0.04 |

The main effects are treatment (T), hour (H) and T × H, with H as a repeated term. Pairwise comparisons of the three treatments versus control were determined *a priori*. Significance of *F* values is given in the table where non-statistical significance (ns) > 0.1. Also shown are mean daily values of $g_s$ for each treatment on each date (± one SE of the mean).
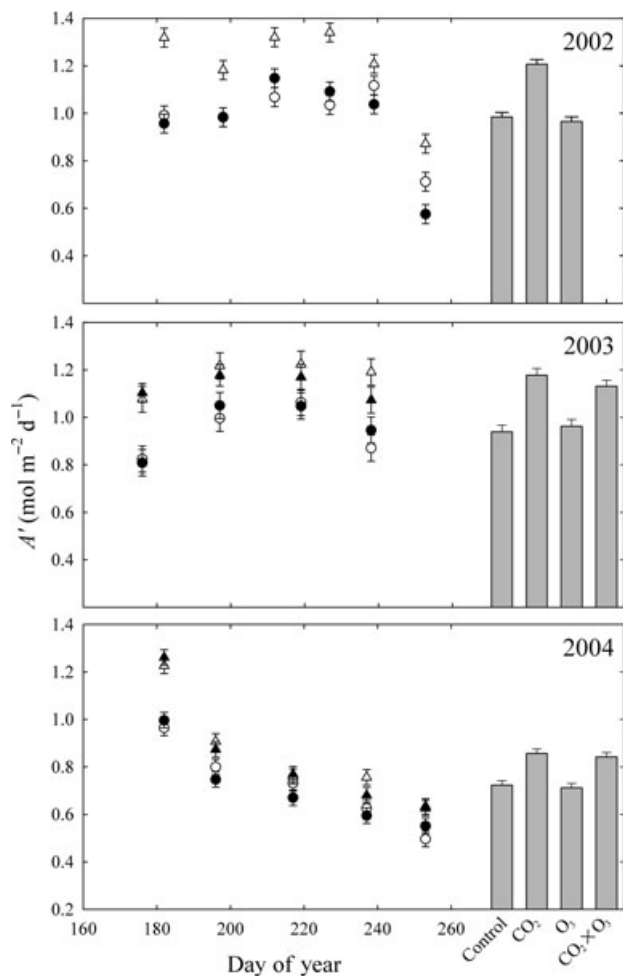
**Figure 4.** Daily integral of carbon assimilation ($A'$) for all measurement dates for all three growing seasons: 2002, 2003 and 2004. Seasonal means of $A'$ for each treatment in each year are also presented as bars to the right in each graph. Open symbols represent ambient [$O_3$], closed symbols represent elevated [$O_3$], circles represent ambient [$CO_2$] and triangles represent elevated [$CO_2$]. Error bars represent one SE around the mean.

however, there was a general and significant increase in $J'$ averaging 5% across the 3 years (Fig. 6, Table 5). No treatment effect of elevated [$O_3$] was observed for any of the 3 years. Seasonal average $J'$ was 4.4% greater in the elevated [$CO_2$] + [$O_3$] treatment than the control treatment in 2004.

To more clearly assess the impacts of the various treatments on $A$, $J_{PSII}$ and $g_s$, and to allow for a more direct comparison of these with previously published reports, midday values were extracted from each diurnal data set (Fig. 7). The stimulation of midday rates of $A$ and $J_{PSII}$ was slightly higher than $A'$ and $J'$, respectively, for all 3 years (Table 5). A decrease in midday $g_s$ with elevated [$CO_2$] and elevated [$CO_2$] + [$O_3$] occurred on a majority of the measurement days (Fig. 7) and resulted in statistically significant decreases throughout all growing seasons (Table 5). However, the responses of midday $g_s$ over the three seasons to elevated [$CO_2$] appear to differ, as 2002 and 2003 showed

a 20 and 23% decrease in midday $g_s$, respectively, whereas 2004 resulted in a 35% decrease (Fig. 7). In parallel to the diurnal response, no effect of elevated [$O_3$] on midday $g_s$ was apparent.

## DISCUSSION

We tested the hypotheses that (1) the response of leaf photosynthesis ($A$), $g_s$ and whole chain photosystem II electron transport ($J_{PSII}$) to elevated [$CO_2$] will be less under fully open-air conditions than previous reports; (2) that responses to [$CO_2$] will be affected by both date and time of day; (3) stimulation of photosynthesis by elevated [$CO_2$] will be greater with higher temperatures and water stress; and (4) no effect of elevated [$O_3$] will be apparent on $A$, $g_s$ and $J_{PSII}$ in newly expanded leaves when compared with control or to elevated [$CO_2$] treatment. Hypothesis 1 was partially supported and hypotheses 2, 3 and 4 were fully supported by the results.
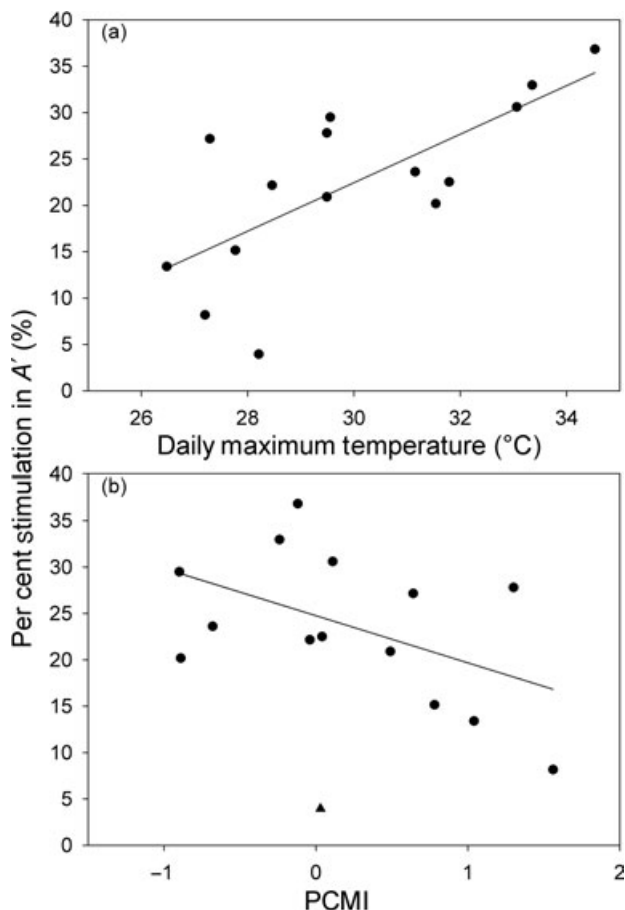


**Figure 5.** The relationship between per cent stimulation in $A'$ in elevated [$CO_2$] compared with control plotted as a function of (a) daily maximum temperature and (b) Palmer crop moisture index (PCMI) for all 3 years combined. The lines represent first-order linear regressions fitted through the data ($r^2 = 0.51$, $P = 0.003$ for panel a and $r^2 = 0.25$, $P = 0.0675$ for panel b). One measurement date, day of year 217 in 2004, was excluded from the linear regression analysis for panel b and is represented by a triangle symbol.

**Table 5.** Statistical analysis of the main effects and pairwise comparisons for midday rates of carbon uptake ($A$), daily integral of $A$ ($A'$), midday rates of whole chain electron transport through photosystem II ($J_{PSII}$), daily integrated $J_{PSII}$ ($J'$) and midday values of stomatal conductance ($g_s$)

| 2002 | Main effects | | | Control versus | | | % Deviation from control | | |
|---|---|---|---|---|---|---|---|---|---|
| | T | D | T × D | $CO_2$ | $O_3$ | $CO_2 + O_3$ | $CO_2$ | $O_3$ | $CO_2 + O_3$ |
| Midday $A$ | < 0.001 | < 0.01 | ns | < 0.01 | ns | – | 24.2 | 5.5 | |
| $A'$ | < 0.001 | < 0.001 | < 0.05 | < 0.001 | ns | – | 22.6 | –1.9 | – |
| Midday $J_{PSII}$ | < 0.01 | ns | ns | < 0.1 | ns | – | 5.1 | 1.7 | |
| $J'$ | < 0.01 | < 0.001 | ns | < 0.05 | ns | – | 4.6 | –1.7 | – |
| Midday $g_s$ | < 0.1 | < 0.001 | ns | < 0.1 | ns | – | –23.7 | –2.4 | – |
| **2003** | | | | | | | | | |
| Midday $A$ | < 0.05 | < 0.001 | ns | < 0.001 | ns | < 0.001 | 30.6 | 1.8 | 29.9 |
| $A'$ | < 0.001 | < 0.01 | ns | < 0.001 | ns | < 0.001 | 25.4 | 2.6 | 20.5 |
| Midday $J_{PSII}$ | < 0.01 | < 0.05 | ns | < 0.05 | ns | < 0.1 | 7.0 | –1.2 | 4.9 |
| $J'$ | < 0.1 | < 0.001 | ns | < 0.05 | ns | ns | 6.7 | 1.9 | 0.3 |
| Midday $g_s$ | < 0.01 | < 0.01 | ns | < 0.01 | ns | < 0.05 | –20.5 | 4.1 | –17.3 |
| **2004** | | | | | | | | | |
| Midday $A$ | < 0.01 | 0.0003 | ns | < 0.001 | ns | < 0.01 | 22.9 | –1.8 | 20.0 |
| $A'$ | < 0.001 | < 0.001 | < 0.01 | < 0.001 | ns | < 0.001 | 18.4 | –1.6 | 16.4 |
| Midday $J_{PSII}$ | < 0.001 | < 0.001 | ns | < 0.01 | ns | < 0.001 | 5.7 | 0.0 | 8.4 |
| $J'$ | < 0.01 | < 0.001 | ns | < 0.05 | ns | < 0.05 | 3.7 | –0.5 | 4.4 |
| Midday $g_s$ | < 0.001 | < 0.05 | ns | < 0.001 | ns | < 0.001 | –36.7 | –4.1 | –30.6 |

The data were analysed using a repeated measures complete-block analysis of variance with treatment (T), hour (H) and T × H as the main effects. *A priori* determined pairwise comparisons of the three treatments versus control and the per cent deviation of the various treatments from control are also shown. Non-statistical significance is denoted by *ns*. Means for the control and treatments are shown in Figs 4, 6 and 7.

Meta-analyses of 111 prior studies of soybean grown in enclosed environments show an increase in $A$ of 39% and a decrease in $g_s$ of 40% at [$CO_2$] elevated on average to 689 $\mu$mol mol$^{-1}$ (Ainsworth *et al.* 2002). Assuming a linear response of these variables to [$CO_2$], this would translate to an increase in $A$ of 20% and a decrease in $g_s$ of 20% with elevation of $CO_2$ to 550 $\mu$mol mol$^{-1}$. In this open-air experiment in which $CO_2$ was elevated to 550 $\mu$mol mol$^{-1}$, $A$ was increased by 24% and $g_s$ decreased by 16%. These suggest similar responses in the two environments. However, assuming a linear response to [$CO_2$] likely underestimates the true response indicating that the open-air responses might still be slightly less than in the enclosure studies.

Elevated [$CO_2$] consistently resulted in slightly higher $J_{PSII}$ throughout the 3 years of measurements (Fig. 6, Tables 3 & 5). Rogers *et al.* (2004), studying a different soybean cultivar in 2001, noted a possible increase in $J_{PSII}$, but this was not significant. Here a statistically significant increase in $J_{PSII}$ is observed. In theory $J_{PSII}$ could increase, decrease, or remain constant with growth in elevated [$CO_2$] depending on whether photosynthesis is limited by Rubisco, RuBP or triose phosphate export from the chloroplast, respectively (Hymus, Baker & Long 2001). We have previously shown that the photosynthetic operating point for control plants grown at SoyFACE is near, or slightly below, the inflection point where photosynthesis is co-limited by Rubisco and RuBP (Bernacchi *et al.* 2005). Despite the slight down-regulation of Rubisco with

elevated [$CO_2$], the shift in the operating point of photosynthesis from Rubisco- to RuBP-limiting without a decrease in photosynthetic potential (Bernacchi *et al.* 2005) supports a slight increase in $J_{PSII}$, but one smaller than the increase in $A$ (Hymus *et al.* 2001) as reported here.

The mean increase in $A$ with elevated [$CO_2$] varied depending on the timescale under consideration. Percentage increases in $A$ for instantaneous measurements ranged from slight decreases to increases of over 50%. However, when integrated over the day, the stimulation of $A'$ by elevated [$CO_2$] ranged from 4 to 37% (Fig. 5). Little or no increase in $A$ with plant growth in elevated [$CO_2$] was apparent at the low-light fluxes of early morning and dusk (Fig. 2) accounting for the significant interaction of time of day and [$CO_2$] (Table 2). This is consistent with the expectation from Rubisco kinetics that $A$ at 25 °C would be stimulated by 37% at light saturation, but only 11% when photosynthesis is light-limited (Long *et al.* 2004). This is because at light saturation, elevated [$CO_2$] both increases the velocity of carboxylation and partially inhibits oxygenation at Rubisco. At low light, $CO_2$ is not limiting the rate of carboxylation but does inhibit oxygenation. As a result, the proportion of RuBP that is oxygenated decreases as [$CO_2$] rises, resulting in a net increase in $CO_2$ uptake (Long *et al.* 2004). This is also consistent with the observed effect of elevated [$CO_2$] on $J_{PSII}$ where increase is evident only around midday when photosynthesis may be limited by the amount of active Rubisco and increased velocity of
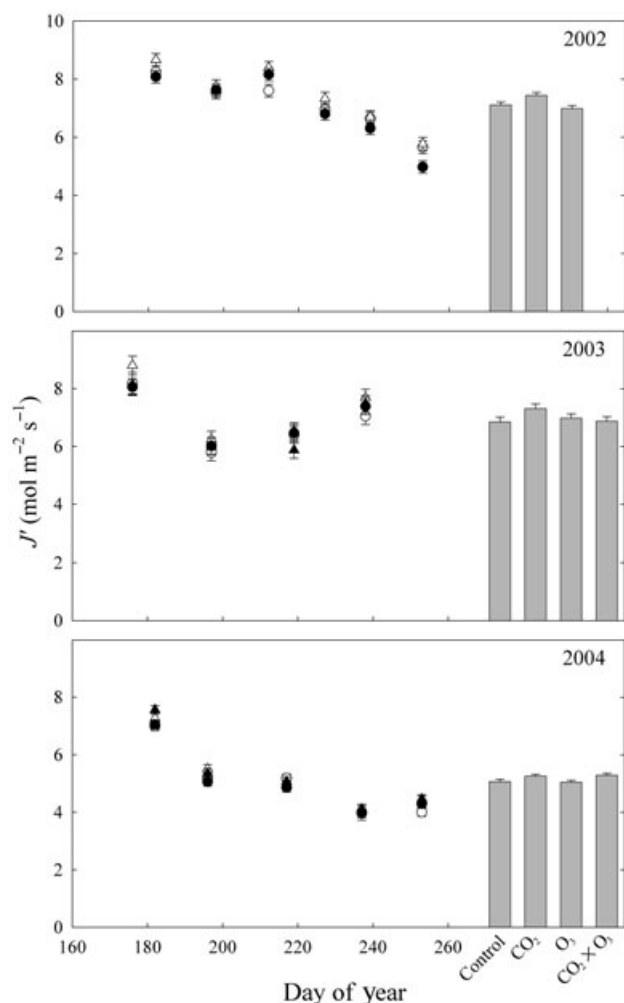
**Figure 6.** Daily integral of electron transport rates ($J'$) for all measurement dates for all three growing seasons: 2002, 2003 and 2004. Seasonal means of $J'$ for each treatment in each year are also presented as bars to the right in each graph. Open symbols represent ambient [$O_3$], closed symbols represent elevated [$O_3$], circles represent ambient [$CO_2$] and triangles represent elevated [$CO_2$]. Error bars represent one SE around the mean.

carboxylation at elevated [$CO_2$] will increase the electron transport requirement. In low light when RuBP is limiting, the electron transport requirement will be unaffected by a change in the ratio of oxygenation/carboxylation (Long & Bernacchi 2003). Elevated [$CO_2$] significantly decreased $g_s$ and the diurnal pattern of decrease was more variable than for the increase in $A$. On all dates, midday $g_s$ was significantly less at elevated [$CO_2$] and not significantly different in elevated [$O_3$] (Table 5). By contrast, $g_s$ measured over the diurnal period was significantly less on only eight of the 15 measurement dates for elevated [$CO_2$] and on two dates for elevated [$O_3$]. Inconsistency between diurnal versus midday $g_s$ was likely caused by smaller or no response in the early morning and around dusk, and also potentially compromised by dew on the leaves in the morning of several of the measurement dates. However, variation in the $CO_2$ effect on $g_s$, during the middle of the diurnal period,

was predictable for dates in 2003 through use of the Ball, Woodrow & Berry (1987) model (Leakey *et al.*, in press). The absence of an elevated [$CO_2$] effect on $g_s$ on specific dates could be explained if it corresponds to periods when soil moisture depletion is reducing $g_s$ in ambient [$CO_2$], but at elevated [$CO_2$] lower whole plant transpiration is conserving soil water and allowing plants to avoid water stress and maintain high $g_s$.

Because both the solubility of $CO_2$ in water and specificity of Rubisco for $CO_2$ decline relative to $O_2$ with rising temperature, the stimulatory effect on $A$ of rising [$CO_2$] should be greater as temperature rises (Long 1991). In practice this theoretical expectation has not been realized in various field studies (e.g. Delgado *et al.* 1994; Naumburg, Loik & Smith 2004). However, here a strong positive correlation between temperature and per cent stimulation of $A$ with growth in elevated [$CO_2$] was observed (Fig. 5). Possibly this was achieved by the statistical sensitivity provided by the very large number of measurements (> 4700) contributing to this analysis. Drought has similarly been expected to result in an increased response to elevated [$CO_2$]. Decreased $g_s$ at elevated [$CO_2$] may conserve soil moisture delaying or avoiding any decrease in assimilation due to decreased leaf and plant water status (Rogers *et al.* 1984; Allen *et al.* 1994, 1998). The PCMI provides an indicator of potential crop water stress for a specific region. Because the reported PCMI values represent a large portion of East Central Illinois, they may not reflect the actual crop moisture status of the SoyFACE field. Nevertheless, the response of $A$ to elevated [$CO_2$] significantly increased during periods of greater water stress (Fig. 5). This corresponds with the observation at SoyFACE of enhanced $A$ in maize during periods of drought stress (Leakey *et al.* 2004). $C_4$ photosynthesis of maize is saturated at ambient [$CO_2$], but lower $g_s$ leads to reduced soil water depletion and the potential for amelioration of drought stress and indirect enhancement of carbon gain (Leakey *et al.* 2006). The same mechanism may well occur in soybean, but is difficult to dissect from the concurrent direct stimulation of $A$ by elevated [$CO_2$].

A meta-analysis of 53 prior chamber studies of soybean in which [$O_3$] was elevated to 70 nmol $mol^{-1}$ (daytime average) showed highly significant reductions in $A$ (20%) and $g_s$ (17%) relative to clean air (< 30 nmol $mol^{-1}$; Morgan *et al.* 2003). Although this first open-air study of soybean under elevated [$O_3$] covers a smaller increase in concentration, not only was there no significant effect of elevation but no change in either $A$ or $g_s$ was indicated. The difference between elevated [$O_3$] effects on $A$ and $g_s$ in this study versus previous experiments may be driven by many factors. Firstly, the meta-analysis focuses on comparisons between [$O_3$] free air and elevated [$O_3$] treatments. Fumigation at SoyFACE is a fixed percentage above the background levels, which represents a smaller differential than the majority of previous studies (Morgan *et al.* 2003). Secondly, it is possible that previous experiments showing an effect of elevated [$O_3$] on plants may overestimate the responses because of forced coupling between the atmosphere and the canopy. The
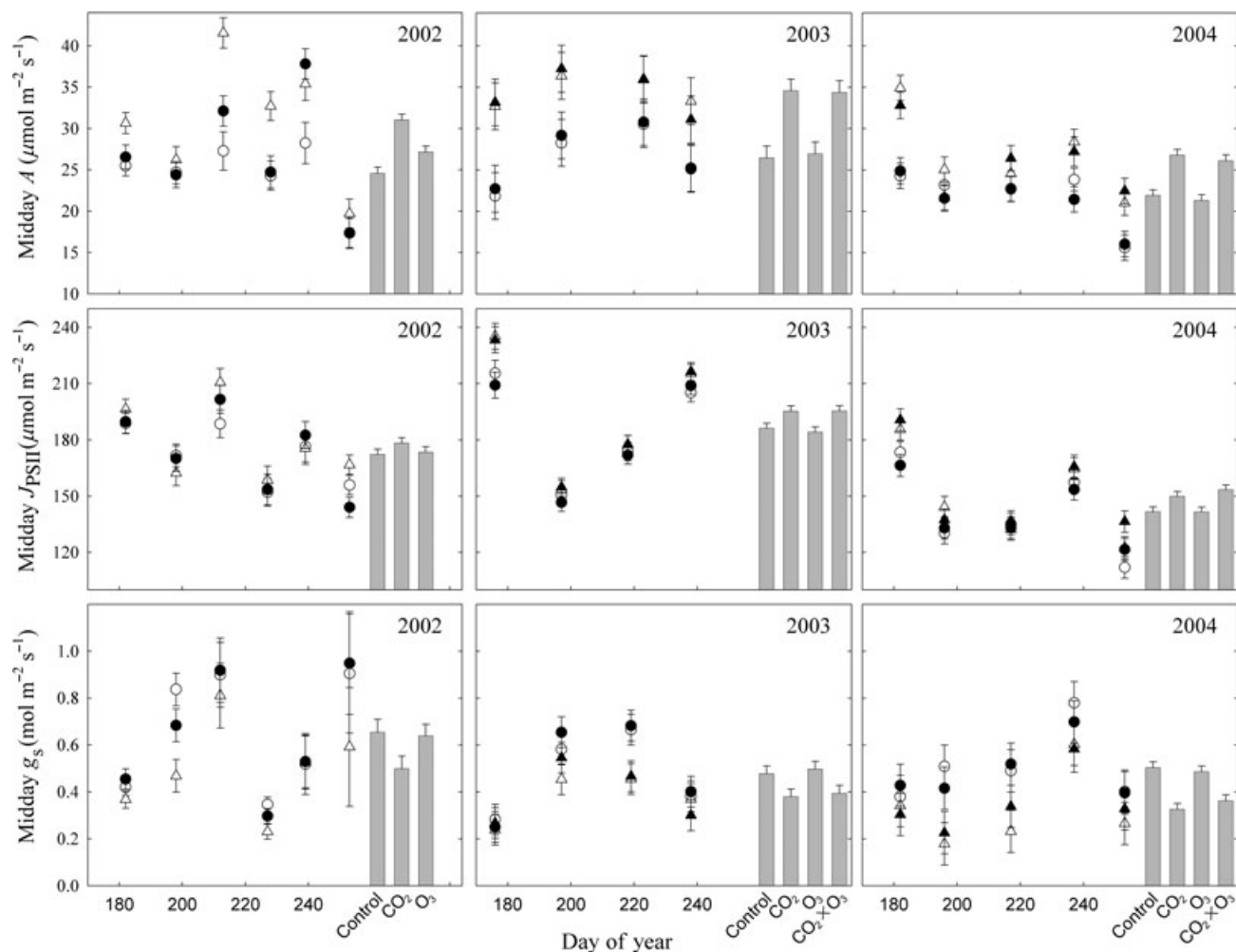
**Figure 7.** Midday measurement of $A$, $J_{PSII}$ and $g_s$ for each measurement period for all three growing seasons: 2002, 2003 and 2004. Data from the measurement period closest to solar noon were used in the analysis. Seasonal mean midday values for $A$, $J_{PSII}$ and $g_s$ are also presented to the right of each graph. Open symbols represent ambient $[O_3]$, closed symbols represent elevated $[O_3]$, circles represent ambient $[CO_2]$ and triangles represent elevated $[CO_2]$. Error bars represent one SE around the mean.

transfer of water from the soil to the bulk atmosphere via transpiration is influenced by at least nine major resistances (e.g. Bazzaz & Sombroek 1996) which are substantially altered in artificial growth environments. The substantial forced mixing that occurs in artificial enclosures reduces many of the canopy resistance pathways, which results in a higher degree of ozone exposure at the leaf level, which would accentuate the effects of ozone. A third possibility may exist in the selection of the youngest fully expanded leaves. As we have previously demonstrated, leaves grown in elevated $[O_3]$ show damage only after prolonged exposure (Morgan *et al.* 2004). By selecting only the recently expanded leaves for this experiment, older leaves in which greater cumulative ozone uptake may have induced damage were avoided. Moreover, it should be noted that despite no obvious effects of elevated $[O_3]$ on photosynthesis, $g_s$ or $J_{PSII}$, significant and consistent decreases in leaf area index (Dermody, Long & DeLucia 2006), total plant biomass and yields (Morgan *et al.* 2005) for soybean grown in elevated $[O_3]$ have been observed in the same plots at SoyFACE.

Although photosynthesis was also increased significantly by elevated $[CO_2]$ even when $[O_3]$ was also elevated, the increase was 19%, 5% less than when $[CO_2]$ was elevated without elevation of $[O_3]$. This resulted from a significant interaction of elevation of $[CO_2]$ and $[O_3]$ on six out of the 15 d of measurements across all three growing seasons.

In conclusion, photosynthesis, $g_s$ and whole chain electron transport through photosystem II all responded to growth in elevated $[CO_2]$ in a predictable manner, although the percentage changes are less than predicted from enclosure studies. Temperature and soil moisture both strongly affect the response of photosynthesis to elevated $[CO_2]$. Elevated $[O_3]$, did not have any effect on recently expanded leaves, although it did slightly diminish the response of photosynthesis to elevated $[CO_2]$.

## ACKNOWLEDGMENTS

## REFERENCES

Ainsworth E.A., Davey P.A., Bernacchi C.J., *et al.* (2002) A meta-analysis of elevated [$CO_2$] effects on soybean (*Glycine max*) physiology, growth and yield. *Global Change Biology* **8,** 695–709.

Ainsworth E.A., Davey P.A., Hymus G.J., Osborne C.E., Rogers A., Blum H., Nosberger J. & Long S.E. (2003) Is stimulation of leaf photosynthesis by elevated carbon dioxide concentration maintained in the long term? A test with *Lolium perenne* grown for 10 years at two nitrogen fertilization levels under Free Air $CO_2$ Enrichment (FACE). *Plant, Cell & Environment* **26,** 705–714.

Ainsworth E.A., Rogers A., Nelson R. & Long S.P. (2004) Testing the 'source-sink' hypothesis of down-regulation of photosynthesis in elevated [$CO_2$] in the field with single gene substitutions in *Glycine max. Agricultural and Forest Meteorology* **122,** 85–94.

Allen L.H., Valle R.R., Mishoe J.W. & Jones J.W. (1994) Soybean leaf gas-exchange responses to carbon-dioxide and water-stress. *Agronomy Journal* **86,** 625–636.

Allen L.H., Valle R.R., Jones J.W. & Jones P.H. (1998) Soybean leaf water potential responses to carbon dioxide and drought. *Agronomy Journal* **90,** 375–383.

Ball J.T., Woodrow I.E. & Berry J.A. (1987) A model predicting stomatal conductance and its contribution to the control of photosynthesis under different environmental conditions. In *Progress in Photosynthesis Research: Proceedings of the VIIth International Congress on Photosynthesis* (ed. J. Biggins), pp. 221–224. Providence, Rhode Island, USA, August 10–15, 1986. Kluwer Academic Publishers, Boston, MA, USA.

Bazzaz F.A. & Sombroek W.G. (1996) *Global Climate Change and Agricultural Production: Direct and Indirect Effects of Changing Hydrological, Pedological, and Plant Physiological Processes.* Food and Agriculture Organization of the United Nations, New York, USA; Wiley, Chichester, UK.

Bernacchi C.J., Morgan P.B., Ort D.R. & Long S.P. (2005) The growth of soybean under free air [$CO_2$] enrichment (FACE) stimulates photosynthesis while decreasing *in vivo* Rubisco capacity. *Planta* **220,** 434–446.

von Caemmerer S. & Farquhar G.D. (1981) Some relationships between the biochemistry of photosynthesis and the gas-exchange of leaves. *Planta* **153,** 376–387.

Delgado E., Mitchell R.A.C., Parry M.A.J., Driscoll S.P., Mitchell V.J. & Lawlor D.W. (1994) Interacting effects of $CO_2$ concentration, temperature and nitrogen supply on the photosynthesis and composition of winter-wheat leaves. *Plant, Cell & Environment* **17,** 1205–1213.

Dermody O., Long S.P. & DeLucia E.H. (2006) How does elevated $CO_2$ or ozone affect the leaf-area index of soybean when applied independently? *New Phytologist* **169,** 145–155.

Elagoz V. & Manning W.J. (2005) Responses of sensitive and tolerant bush beans (*Phaseolus vulgaris* L.) to ozone in open-top chambers are influenced by phenotypic differences, morphological characteristics and the chamber environment. *Environmental Pollution* **136,** 371–383.

Farquhar G.D., Caemmerer S.V. & Berry J.A. (1980) A biochemical model of photosynthic $CO_2$ assimilation in leaves of $C_3$ species. *Planta* **149,** 78–90.

Garcia R.L., Long S.P., Wall G.W., Osborne C.P., Kimball B.A., Nie G.Y., Pinter P.J., Lamorte R.L. & Wechsung F. (1998) Photosynthesis and conductance of spring-wheat leaves: field response to continuous free-air atmospheric $CO_2$ enrichment. *Plant, Cell & Environment* **21,** 659–669.

Genty B., Briantais J.M. & Baker N.R. (1989) The relationship between the quantum yield of photosynthetic electron-transport and quenching of chlorophyll fluorescence. *Biochimica et Biophysica Acta* **990,** 87–92.

Hymus G.J., Baker N.R. & Long S.P. (2001) Growth in elevated $CO_2$ can both increase and decrease photochemistry and photoinhibition of photosynthesis in a predictable manner. *Dactylis glomerata* grown in two levels of nitrogen nutrition. *Plant Physiology* **127,** 1204–1211.

IPCC (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, Cambridge, UK.

Keselman H.J., Algina J., Kowalchuk R.K. & Wolfinger R.D. (1998) A comparison of two approaches for selecting covariance structures in the analysis of repeated measurements. *Communications in Statistics-Simulation and Computation* **27,** 591–604.

Lawlor D.W. & Mitchell R.A.C. (1991) The effects of increasing $CO_2$ on crop photosynthesis and productivity – a review of field studies. *Plant, Cell & Environment* **14,** 807–818.

Leakey A.D.B., Press M.C., Scholes J.D. & Watling J.R. (2002) Relative enhancement of photosynthesis and growth at elevated CO2 is greater under sunflecks than uniform irradiance in a tropical rain forest tree seedling. *Plant, Cell & Environment* **25,** 1701–1714.

Leakey A.D.B., Bernacchi C.J., Dohleman F.G., Ort D.R. & Long S.P. (2004) Will photosynthesis of maize (*Zea mays*) in the US Corn Belt increase in future [CO2] rich atmospheres? An analysis of diurnal courses of $CO_2$ uptake under free-air concentration enrichment (FACE). *Global Change Biology* **10,** 951–962.

Leakey A.D.B., Uribelarrea M., Ainsworth E.A., Naidu S.L., Rogers A., Ort D.R. & Long S.P. (2006) Photosynthesis, productivity and yield of maize are not affected by open-air elevation of $CO_2$ concentration in the absence of drought. *Plant Physiology* **140,** 779–790.

Leakey A.D.B., Bernacchi C.J., Ort D.R. & Long S.P. Long-term growth of soybean at elevated [CO2] does not cause acclimation of stomatal conductance under fully open-air conditions. *Plant, Cell & Environment* (in press).

Littell R.C., Henry P.R. & Ammerman C.B. (1998) Statistical analysis of repeated measures data using SAS procedures. *Journal of Animal Science* **76,** 1216–1231.

Littell R.C., Pendergast J. & Natarajan R. (2000) Modelling covariance structure in the analysis of repeated measures data. *Statistics in Medicine* **19,** 1793–1819.

Long S.P. (1991) Modification of the response of photosynthetic productivity to rising temperature by atmospheric $CO_2$ concentrations – has its importance been underestimated? *Plant, Cell & Environment* **14,** 729–739.

Long S.P. & Bernacchi C.J. (2003) Gas exchange measurements, what can they tell us about the underlying limitations to photosynthesis? Procedures and sources of error. *Journal of Experimental Botany* **54,** 2393–2401.

Long S.P. & Naidu S.L. (2002) Effects of oxidants at the biochemical, cell and physiological levels. In *Air Pollution and Plant Life* (eds J.N.B. Bell & M. Treshow), pp. 69–88. John Wiley & Sons, New York, USA.

Long S.P., Ainsworth E.A., Rogers A. & Ort D.R. (2004) Rising atmospheric carbon dioxide: plants face the future. *Annual Review of Plant Biology* **55,** 591–628.

Luo Y.Q., Sims D.A. & Griffin K.L. (1998) Nonlinearity of photosynthetic responses to growth in rising atmospheric $CO_2$: an experimental and modelling study. *Global Change Biology* **4,** 173–183.

Mauzerall D.L. & Wang X.P. (2001) Protecting agricultural crops from the effects of tropospheric ozone exposure: reconciling science and standard setting in the United States, Europe and Asia. *Annual Review of Energy and the Environment* **26,** 237–268.

Mckee I.F., Farage P.K. & Long S.P. (1995) The interactive effects of elevated $CO_2$ and $O_3$ concentration on photosynthesis in spring wheat. *Photosynthesis Research* **45,** 111–119.

Miglietta F., Peressotti A., Vaccari F.P., Zaldei A., deAngelis P. & Scarascia-Mugnozza G. (2001) Free-air $CO_2$ enrichment (FACE) of a poplar plantation: the POPFACE fumigation system. *New Phytologist* **150,** 465–476.

Morgan P.B., Ainsworth E.A. & Long S.P. (2003) How does elevated ozone impact soybean? A meta-analysis of photosynthesis, growth and yield. *Plant, Cell & Environment* **26,** 1317–1328.

Morgan P.B., Bernacchi C.J., Ort D.R. & Long S.P. (2004) An *in vivo* analysis of the effect of season-long open-air elevation of ozone to anticipated 2050 levels on photosynthesis in soybean. *Plant Physiology* **135,** 2348–2357.

Morgan P.B., Mies T.A., Bollero G.A., Nelson R.L. & Long S.P. (2005) Season-long elevation of ozone concentration to projected 2050 levels under fully open-air conditions substantially decreases the growth and production of soybean. *New Phytologist* **170,** 333–343.

Naumburg E., Loik M.E. & Smith S.D. (2004) Photosynthetic responses of *Larrea tridentata* to seasonal temperature extremes under elevated $CO_2$. *New Phytologist* **162,** 323–330.

Palmer W.C. (1968) Keeping track of crop moisture conditions, nationwide: the new Crop Moisture Index. *Weatherwise* **21,** 156–161.

Pell E.J., Eckardt N. & Enyedi A.J. (1992) Timing of ozone stress and resulting status of ribulose bisphosphate carboxylase-oxygenase and associated net photosynthesis. *New Phytologist* **120,** 397–405.

Polley H.W. (2002) Implications of atmospheric and climatic change for crop yield and water use efficiency. *Crop Science* **42,** 131–140.

Prather M., Ehhalt D., Dentener F., *et al.* (2001) Atmospheric chemistry and greenhouse gases. 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 J.T. Houghton, Y. Ding, D.J. Griggs, M. Noguer, P.J. van der Linder, X. Dai, K. Maskell, & C.A. Johnson), pp. 239–287. Cambridge University Press, Cambridge, UK and New York, USA.

Prather M., Gauss M., Berntsen T., *et al.* (2003) Fresh air in the 21st century? *Geophysical Research Letters* **30,** 1100.

Reid C.D., Fiscus E.L. & Burkey K.O. (1998) Combined effects of chronic ozone and elevated $CO_2$ on Rubisco activity and leaf components in soybean (*Glycine max*). *Journal of Experimental Botany* **49,** 1999–2011.

Ritchie S.W., Hanway J.J., Thompson E. & Benson G.O. (1989) *How a soybean plant develops*. Special Report No. 53, Iowa State University of Science and Technology, Cooperative Extension Service.

Rogers H.H., Sionit N., Cure J.D., Smith J.M. & Bingham G.E. (1984) Influence of elevated carbon dioxide on water relations of soybeans. *Plant Physiology* **74,** 233–238.

Rogers A., Allen D.J., Davey P.A., *et al.* (2004) Leaf photosynthesis and carbohydrate dynamics of soybeans grown throughout their life-cycle under Free-air Carbon Dioxide Enrichment. *Plant, Cell & Environment* **27,** 449–458.

Vu J.C.V., Allen L.H., Boote K.J. & Bowes G. (1997) Effects of elevated $CO_2$ and temperature on photosynthesis and Rubisco in rice and soybean. *Plant, Cell & Environment* **20,** 68–76.

Xu D.Q., Gifford R.M. & Chow W.S. (1994) Photosynthetic acclimation in pea and soybean to high atmospheric $CO_2$ partial-pressure. *Plant Physiology* **106,** 661–671.

## SUPPLEMENTARY MATERIAL

The following supplementary material is available for this article:

**Figure S1.** Meteorological data for each of the 15 measurement days during the 3 year experiment. Data for air temperature (open circles), VPD (ticks) and photosynthetically active radiation flux (PAR, lines) are presented in 10 min averages of 10 s measurements from a weather station located at SoyFACE.

**Figure S2.** Measurements of leaf-level photosynthesis ($A$) as a function of time of day over the complete diurnal cycle for all 15 measurement days over the 3 year experiment. Each panel represents one measurement day. Each point is the mean of four replicate plots with three subsamples per plot. Open symbols represent ambient and closed symbols elevated [$O_3$] and circles represent control [$CO_2$] and triangles elevated [$CO_2$]. Error bars are one SD around the mean.

**Figure S3.** Measurements of leaf-level whole chain electron transport through photosystem II ($J_{PSII}$) as a function of time of day over the complete diurnal cycle for all 15 measurement days over the 3 year experiment. Each panel represents one measurement day. Each point is the mean of four replicate plots with three subsamples per plot. Open symbols represent ambient and closed symbols elevated [$O_3$] and circles represent control [$CO_2$] and triangles elevated [$CO_2$]. Error bars are one SD around the mean.

**Figure S4.** Measurements of stomatal conductance ($g_s$) as a function of time of day over the complete diurnal cycle for all 15 measurement days over the 3 year experiment. Each panel represents one measurement day. Each point is the mean of four replicate plots with three subsamples per plot. Open symbols represent ambient and closed symbols elevated [$O_3$] and circles represent control [$CO_2$] and triangles elevated [$CO_2$]. Error bars are one SD around the mean.

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