

How does elevated CO₂ or ozone affect the leaf-area index of soybean when applied independently?

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Summary

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- Changes in leaf-area index (LAI) may alter ecosystem productivity in elevated [CO₂] or [O₃]. By increasing the apparent quantum yield of photosynthesis ($\phi_{c,max}$), elevated [CO₂] may increase maximum LAI. However, [O₃] when elevated independently accelerates senescence and may reduce LAI.
- Large plots (20 m diameter) of soybean (*Glycine max*) were exposed to ambient (approx. 370 $\mu\text{mol mol}^{-1}$) or elevated (approx. 550 $\mu\text{mol mol}^{-1}$) CO₂ or 1.2 times ambient [O₃] using soybean free-air concentration enrichment (SoyFACE).
- In 2001 elevated CO₂ had no detectable effect on maximum LAI, but in 2002 maximum LAI increased by 10% relative to ambient air. Elevated [CO₂] also increased the $\phi_{c,max}$ of shade leaves in both years. Elevated [CO₂] delayed LAI loss to senescence by approx. 54% and also increased leaf-area duration. Elevated [O₃] accelerated senescence, reducing LAI by 40% near the end of the growing season. No effect of elevated [O₃] on photosynthesis was detected.
- Elevated [CO₂] or [O₃] affected LAI primarily by altering the rate of senescence; knowledge of this may aid in optimizing future soybean productivity.

Key words: leaf-area index (LAI), senescence, soybean (*Glycine max*), SoyFACE (soybean free-air concentration enrichment).

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Introduction

Plant canopies represent an interface for material and energy exchange between the atmosphere and the terrestrial biosphere, and leaf area and orientation within the canopy determine ecosystem capacity for light interception and productivity. Thus leaf-area index (LAI), the amount of leaf area per unit ground area, is a key parameter in modeling the response of global productivity to atmospheric change (Cowling & Field, 2003; Ewert, 2004). Predicted increases in global [CO₂] (Prentice *et al.*, 2001) and tropospheric [O₃] (Prather *et al.*, 2001) are likely to have contrasting effects on LAI. While elevated [CO₂] generally stimulates photosynthesis (Drake *et al.*, 1997; Ainsworth *et al.*, 2002; Long *et al.*, 2004), elevated [O₃] often has the opposite effect (Fuhrer, 2003; Morgan *et al.*, 2003). Understanding how these gases influence LAI will be important for predicting ecosystem responses to key elements of global change.

Elevated CO₂ may increase LAI by at least two mechanisms. By increasing photosynthetic efficiency, elevated [CO₂] will lower the light-compensation point (LCP) of photosynthesis, allowing leaves to maintain a positive carbon balance in deeper shade than at present atmospheric [CO₂] (Percy, 1983; Long, 1991; Long & Drake, 1991; Hirose *et al.*, 1996). Additionally, greater carbohydrate supply and improved water-use efficiency may lead to larger individual leaves and more rapid canopy development, thereby increasing both maximum LAI and LAI at other points in the growing season (Pritchard *et al.*, 1999; Ferris *et al.*, 2001).

Despite these expectations, quantitative reviews of previous studies have failed to find consistent increases in LAI. In one review, Drake *et al.* (1997) found that, averaged across previous experiments, elevated [CO₂] had no effect on LAI. In a review of free-air concentration enrichment (FACE) experiments applied to large treatment plots (>8 m diameter), Long *et al.* (2004) found that, on average, there was a small but not

statistically significant increase in the LAI of plots under elevated $[\text{CO}_2]$. Based on results derived from chamber experiments, Cowling & Field (2003) concluded that there was no response of crop LAI to elevated $[\text{CO}_2]$; but a meta-analysis of soybean (*Glycine max*) responses to elevated $[\text{CO}_2]$ revealed that, on average, LAI increased by 18% relative to ambient air (Ainsworth *et al.*, 2002).

Low nitrogen availability has the potential to limit the stimulation of LAI by elevated $[\text{CO}_2]$ (Arnone & Körner, 1995; Hirose *et al.*, 1996; Hartz-Rubin & DeLucia, 2001; Franklin & Ågren, 2002). When limiting to growth, N may be remobilized from older tissues to developing leaves in elevated $[\text{CO}_2]$, potentially accelerating senescence of shaded foliage and reducing LAI (Hirose *et al.*, 1996; Hartz-Rubin & DeLucia, 2001). Despite their capacity to fix N, legumes have not shown a significantly greater response to elevated $[\text{CO}_2]$ than other C_3 plants across different FACE experiments (Long *et al.*, 2004). Most importantly, Pinter *et al.* (2000) found no significant increase in LAI of wheat grown under FACE with a high level of N fertilization, although, this could reflect the strongly determinate nature of modern wheat cultivars, restricting any potential increase in LAI. These findings raise the question of whether a lack of increase in LAI with growth in elevated $[\text{CO}_2]$ is a general phenomenon, or a result of restricted growth caused by environmental or genetic factors.

While an increase in LAI with elevated $[\text{CO}_2]$ is uncertain, decreased leaf area is a consistent feature of plants exposed to elevated $[\text{O}_3]$. When grown at elevated background O_3 levels, leaves may mature similarly to controls, but progressively lose photosynthetic capacity and senesce more rapidly (Pell *et al.*, 1997; Miller *et al.*, 1998; Sandermann *et al.*, 1998; Ewert & Pleijel, 1999; Isebrands *et al.*, 2001). While senescence is accelerated, formation of new leaves may be slowed by decreased supply of assimilates and translocation (Grantz & Farrar, 1999; Morgan *et al.*, 2003). Soybeans are particularly sensitive to O_3 (Murphy *et al.*, 1999; Morgan *et al.*, 2003). On contact with plant surfaces O_3 elicits a characteristic stress response, including the emission of ethylene and jasmonate, affecting leaf development and longevity (Long & Naidu, 2002). The inability of older leaves to maintain a positive carbon balance may further accelerate senescence deep in the canopy and reduce LAI (Pell *et al.*, 1997; Miller *et al.*, 1998; Sandermann *et al.*, 1998; Ewert & Pleijel, 1999; Isebrands *et al.*, 2001).

As with elevated $[\text{CO}_2]$, previous studies of the effects of $[\text{O}_3]$ on soybeans have been conducted in enclosures (Morgan *et al.*, 2003), potentially causing an overestimate of the effects of $[\text{O}_3]$ on LAI. By increasing humidity around the leaf, growth in an enclosure may increase stomatal opening and O_3 uptake. Furthermore, the forced circulation of air within chambers may increase exposure of lower canopy leaves to the bulk atmosphere, thus artificially increasing O_3 uptake by shaded foliage. In contrast to previous chamber studies using

similar levels of fumigation, Morgan *et al.* (2004) found that under fully open air $[\text{O}_3]$ fumigation, there was no significant effect of a season-long 20% elevation of $[\text{O}_3]$ on photosynthesis of leaves on completion of expansion: photosynthetic capacity was reduced only in older leaves at the top of the canopy during late grain filling of the crop. This raises the question whether the large decreases in LAI observed in chamber studies may overestimate the effect under open-air conditions in the field.

In 2004, soybeans were planted on 30.4 million ha in the USA, and the total crop value exceeded US\$17.7 billion. Elevated $[\text{CO}_2]$ may improve these figures, but given soybean sensitivity to elevated $[\text{O}_3]$, the value of the crop may decline with future projected increases in tropospheric $[\text{O}_3]$. The mid-west is a major centre of soybean growth and is also exposed to some of the highest background O_3 levels worldwide (Prather *et al.*, 2001). Because of this, the value of the national soybean crop will be particularly susceptible to future increases in tropospheric $[\text{O}_3]$.

The objective of this research was to quantify the effect of elevated $[\text{CO}_2]$ or $[\text{O}_3]$, applied independently, on the seasonal dynamics of LAI in a soybean canopy. Large plots (20 m diameter) in a soybean field were exposed to elevated levels of CO_2 or O_3 with soybean free-air concentration enrichment (SoyFACE) technology. At the time of this research a combined elevated $[\text{CO}_2]$ plus $[\text{O}_3]$ treatment was not available. We hypothesized that elevated $[\text{CO}_2]$ would increase maximum LAI. It was expected that increased LAI would occur with an increase in the maximum quantum efficiency of photosynthesis ($\phi_{c,\text{max}}$) and lower LCP of shade leaves in elevated $[\text{CO}_2]$. Additionally, we expected that increased maximum LAI in elevated $[\text{CO}_2]$ would be maintained in part by remobilization of N from older to developing leaves. We also hypothesized that elevated $[\text{O}_3]$ would reduce LAI primarily at the end of the season by accelerating senescence; this reduction in LAI would then lead to lower leaf-area duration (LAD).

Materials and Methods

Site description

This study was conducted at the SoyFACE facility established in 2001 at the University of Illinois, Urbana-Champaign (40°02' N, 88°14' W, 228 m above sea level; <http://www.soyface.uiuc.edu>). Each experimental plot was circumscribed by a segmented octagon of pipes that injected CO_2 or O_3 at a supersonic velocity from 300- μm pores above the soybean canopy (Miglietta *et al.*, 2001). The rate and position of gas release was automatically and continuously altered with wind speed and direction to maintain the desired enrichment within the plot. In 2001 and 2002, the target for the elevated $[\text{CO}_2]$ plots was 550 $\mu\text{mol mol}^{-1} \text{CO}_2$; the actual average concentrations were 549 and 552 $\mu\text{mol mol}^{-1}$, respectively, while the ambient plots were approx. 370 $\mu\text{mol mol}^{-1} \text{CO}_2$.

In 2002, an elevated [O₃] treatment was added by maintaining experimental plots at 1.5 × current levels. Because fumigation was stopped when leaf surfaces were damp, the actual seasonal dose was 20% above ambient concentrations. At current rates of anthropogenic emissions, the targets for both [CO₂] and [O₃] represent predicted atmospheric levels by 2050 (Prather *et al.*, 2001; Prentice *et al.*, 2001).

Plants were fumigated during daylight hours for the entire growing season. One-minute average [CO₂] and [O₃] were ±20% of the target for >95% of the time. The experimental plots were separated by at least 100 m to prevent cross-contamination of CO₂ and O₃ (Nagy *et al.*, 1994). A more detailed description of the SoyFACE facility is given by Rogers *et al.* (2004).

In 2001 the experiment consisted of four randomized blocks, each containing two 20-m-diameter octagonal plots (total area 282.8 m²). Within each block, one plot was maintained at ambient [CO₂] and one at elevated [CO₂]. An elevated [O₃] plot was added to each block in 2002. The combined elevated [CO₂] plus [O₃] treatment was not available at this time. Soybean (*Glycine max* L. cv. Pana, Illinois Foundation Seeds, Champaign, IL, USA) was planted at 0.38-m row spacing in May 2001. This cultivar was taller than average and fell over (lodged) during windy conditions in mid-August 2001. Because of lodging, the genotype was changed to a closely related but shorter cultivar in 2002 (cv. 93B15, Pioneer Hi-Bred, Johnston, IA, USA). Plants were not inoculated with *Bradyrhizobium* because those bacteria are ubiquitous in Illinois soils that have had soybeans in the crop rotation. The varieties were in maturity group III and were resistant to soybean cyst nematode. Apart from not being Roundup Ready, these varieties are typical of those grown in commercial production.

The soil was deep and fertile, composed of mostly Flanagan (fine, montmorillonitic, mesic aquic Argiudoll). Blocking of the experimental plots reflected differences in soil type, with some blocks composed of Drummer (typic Haplaquoll) formed from loess and silt parent material deposited on till and outwash plains (Rogers *et al.*, 2004). According to standard agronomic practice in this region, soybean was also rotated annually with corn and no N fertilizer was added.

Leaf-area index and canopy structure

Leaf-area index was measured at weekly intervals during the summers of 2001 and 2002 with a plant canopy analyzer (LAI-2000, Li-Cor, Lincoln, NE, USA). The plant-canopy analyser estimated LAI through measurements of gap frequency, the probability that a light ray would not contact vegetation as it passed through the canopy (Lang, 1991; Welles & Norman, 1991). The LAI was measured on cloudy days or within 1 h of sunset when the incident light was diffuse. In 2001 LAI was measured at eight random locations within each plot, and in 2002 at six locations. These locations

spanned an area of the plot reserved for LAI and protected from destructive sampling. At each sampling location one measurement above the canopy was paired with four measurements below it, spanning a single row. A view cap was used to exclude the operator, and the entire hemispherical view was used to calculate LAI. To verify the accuracy of the optical method, leaf area also was measured by harvesting small subplots (0.23 m²) bi-weekly. The profile of LAI was also measured on 7 August 2002 at peak LAI, and on 16 September, leaves were removed at 20-cm (2001) or 10-cm (2002) height intervals, then immediately passed through a photodiode-based leaf-area meter (LI-3100, Li-Cor).

Canopy height and depth were measured weekly on six randomly chosen plants in each plot. Canopy depth was defined as the height (cm) of the lowest green leaf subtracted from the height of the highest green leaf on each plant. Lodging of the crop towards the end of the 2001 growing season prevented accurate determination of leaf position with height. Lodging damage was less pronounced with the shorter cultivar in 2002. Leaf-area duration, which represents the duration of canopy area, was calculated for each experimental plot as the area under the curve defined by plotting LAI vs time (Norby *et al.*, 2003). The relationship between LAI and time was fitted using the trapezoidal rule (SIGMAPLOT ver. 7, Systat Software Inc., Point Richmond, CA, USA).

Leaf phenology and chemistry

The dates of birth and death of six leaf cohorts (node numbers 1, 3, 7, 10, 13 and 17) were monitored on two randomly selected plants in each plot throughout the 2002 growing season. Birth was defined as the day when leaflets in a trifoliate no longer touched (Ritchie *et al.*, 1997); senescence was defined as when the leaf was either >85% yellow or had abscised. Leaves were observed once a week. Because we were not able to observe leaves daily, it was necessary in some cases to estimate birth and senescence dates within 2 d of the measurement date.

To determine if N remobilization over the season was altered by elevated [CO₂] or [O₃], two representative leaf discs from each cohort were sampled weekly and dried for determination of leaf N content and specific leaf area (SLA, leaf area per unit dry matter). When leaves were missing or damaged by herbivory, the closest undamaged leaf at the same nodal position on a different plant was sampled. At the beginning of the season 20 plants were marked, and on each plant the cohorts were tagged as they developed. No leaf was sampled more than once. The two leaf discs from individual leaves in each experimental plot were pooled, resulting in a sample size of four. Leaf N content was determined with an elemental CHN analyzer (ECS 4010, COSTECH Analytical Instruments, Valencia, CA, USA). To calculate leaf N content on an area basis, the mass of each sample was multiplied by the SLA of that tissue.

Photosynthesis

To determine if elevated $[\text{CO}_2]$ or $[\text{O}_3]$ altered $\phi_{\text{c,max}}$, the response of net photosynthesis of shade leaves (approx. 40 cm height, one leaf per plot, $n = 4$) to variation in incident irradiation was measured at growth $[\text{CO}_2]$ on 20 July 2001 and 11 July 2002. These days were chosen because they had similar weather. Because we were interested primarily in the shade response, we did not measure sun leaves in 2002. Plants were approx. 36 cm tall in 2001, and approx. 31 cm tall in 2002. The LAI on these dates was approx. 2.3 in 2002 and 2.7 in 2001; average ambient temperature was 20.0°C in 2001 and 20.9°C in 2002; average daily relative humidity was 71% in 2001 and 54% in 2002. On both dates plants were still at the vegetative stage and had not flowered.

Gas exchange was measured *in situ* with a portable open-flow infrared gas analyzer with an attached red-blue LED light source (LI-6400, Li-Cor). Measurements were made on a single leaf per plot for sun and shade. Before the first measurement, the leaf was acclimatized for approx. 15 min to saturating irradiance of 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetic photon flux density. Irradiance was reduced incrementally from 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$, and included at least eight measurements between 10 and 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The use of decreasing light levels reduced the equilibrium time required for stomatal opening and photosynthetic induction (Kubiske & Pregitzer, 1996). Flow rates were fixed and relative humidity was varied between 65 and 80%. The temperature in the gas-exchange cuvette was controlled between 30 and 32°C (similar to ambient temperature).

The nonrectangular hyperbolic relationship (equation 1) of net photosynthesis (A) to photon flux density (Q) was fitted by an iterative maximum-likelihood procedure (SIGMAPLOT ver. 7):

$$A = (Q \times \phi_{\text{c,max}} + A_{\text{sat}} - \{[(\phi_{\text{c,max}} \times Q) + A_{\text{sat}}]^2 - [4 \times \phi_{\text{c,max}} \times A_{\text{sat}} \times Q \times \Theta]\}^{0.5}) / 2\Theta + R_{\text{d}} \quad \text{Eqn 1}$$

according to Leverenz & Oquist (1987) and Leverenz (1995). The maximum apparent quantum yield ($\phi_{\text{c,max}}$) was calculated independently as the slope of the strictly linear light-limited portion of the photosynthetic light-response curve (Long & Drake, 1991). The light-saturated rate of net CO_2 uptake was A_{sat} ; Θ described the convexity of the transition from light-limited to light-saturated photosynthesis; and R_{d} was the dark respiration rate. Occasionally, the Kok effect, defined as an increase in mitochondrial respiration at low irradiance, increased the initial slope of the light-response curve (Kok, 1948). This occurred in <25% of all light curves, and when it occurred the lowest three points were excluded from the analysis, as in Singaas *et al.* (2001). For each curve the specific value of $\phi_{\text{c,max}}$ and A_{sat} were substituted into the above equation for determination of Θ and R_{d} . Dark respiration rate was estimated from the equation because gas exchange was

not measured in complete darkness. Light compensation points were calculated by determining at what light level net CO_2 exchange was equal to 0.

Statistical analysis

After verifying that data fulfilled the assumptions of ANOVA, a repeated measures ANOVA (PROC MIXED: SAS ver. 8.1, SAS Institute, Cary, NC, USA) was used to test for treatment effects on LAI and canopy depth. Analyses were performed on the plot means; date and treatment were fixed effects; and blocks were included as a random component. To determine if the treatment affected the leaf-area profile in August at maximum LAI, and in September when the canopy was senescing, layers were analyzed with a mixed model ANOVA (PROC MIXED) with treatment and layers as fixed effects and blocks as a random component. Photosynthetic parameters were separated by year and analyzed in a mixed model ANOVA (PROC MIXED) with treatment and layers as fixed effects and blocks as a random component. The specific leaf area and N content of cohorts were analyzed in a mixed model ANOVA. *Post hoc* linear contrasts were performed to elucidate treatment effects within interaction terms. All comparisons were made between treatments and control. To avoid type II errors, differences were considered significant at $P = 0.1$.

Results

As initially demonstrated by Welles & Norman (1991), estimates of LAI made with the plant canopy analyzer were highly correlated with values derived from destructive harvests ($\text{LAI}_{\text{optical}} = -1.35 \times \text{LAI}_{\text{harvest}} + 0.075$, $r^2 = 0.96$, $P = 0.01$), and the slopes of regressions between the two methods were statistically indistinguishable from a one-to-one line.

Leaf area developed rapidly during the summer, with the maximum LAI occurring in the first week of August (day 215–222, Fig. 1). Maximum LAI persisted for 1 wk, after which pod fill was initiated and LAI declined. In 2001 we were unable to detect an effect of elevated $[\text{CO}_2]$ on maximum LAI ($F = 0.61$, $P = 0.27$, $n = 4$; Fig. 1). In 2002 elevated $[\text{CO}_2]$ significantly increased LAI 3 wk before maximum LAI, and this stimulation persisted until the end of the season (at maximum LAI, $F = 8.7$, $P = 0.05$, $n = 4$). Exposure to elevated $[\text{CO}_2]$ delayed senescence in 2001 and 2002 (e.g. on day 260 in 2002, $F = 4.2$, $P = 0.04$, $n = 4$), and because of this delay LAD was significantly greater in plots exposed to elevated $[\text{CO}_2]$ [mean 2001, 410 ± 27.8 (SE), $F = 5.3$, $P = 0.06$, $n = 4$; mean 2002, 427 ± 8.2 , $F = 13.5$, $P = 0.002$, $n = 4$] than in ambient air (mean 2001, 356 ± 10.6 ; mean 2002, 398 ± 8.7). Exposure to elevated $[\text{O}_3]$ in 2002 had no effect on maximum LAI ($F = 1.2$, $P = 0.35$, $n = 4$). However, elevated $[\text{O}_3]$ significantly accelerated leaf senescence at the end of the 2002 season (Fig. 1b). Consequently, LAD was lower in plots exposed to elevated $[\text{O}_3]$ than in those

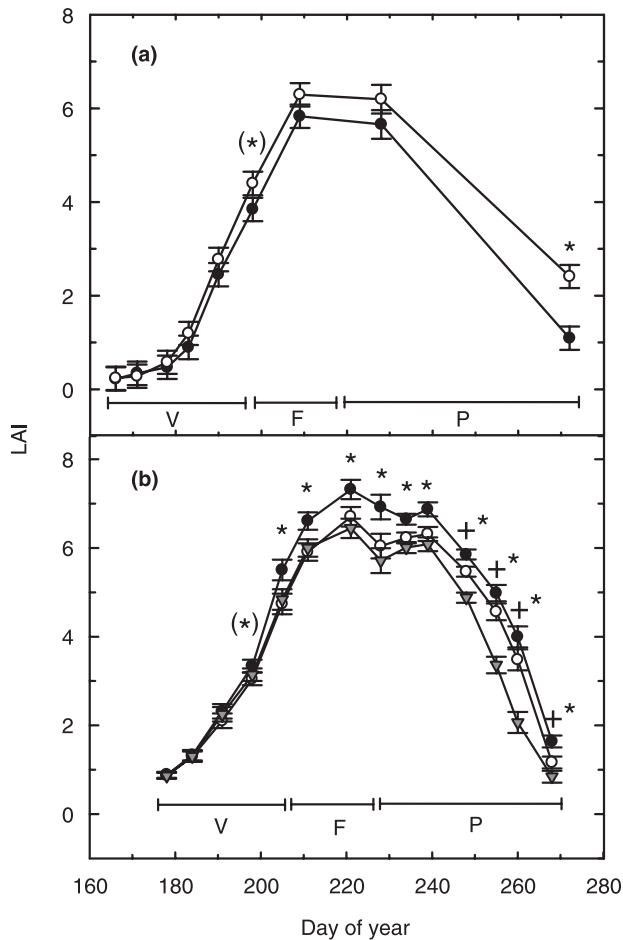


Fig. 1 Leaf-area index (LAI) measured with a plant canopy analyzer for soybean (*Glycine max*) plants grown in ambient air (open circles); $550 \mu\text{mol mol}^{-1} \text{CO}_2$ (closed circles); $1.2 \times$ ambient $[\text{O}_3]$ (grey triangles). In 2001 only ambient and elevated $[\text{CO}_2]$ treatments were present (a); the elevated $[\text{O}_3]$ treatment was added in 2002 (b). Each point represents least-squared mean \pm SE ($n = 4$) calculated from repeated measures ANOVA. Developmental stages of soybeans indicated at the bottom of each graph: V, vegetative; F, flowering; P, pod filling. 15 June, July, August and September correspond to days 167, 197, 228 and 259, respectively. *, +, Significant differences ($P = 0.05$) between elevated $[\text{CO}_2]$ or $[\text{O}_3]$ and ambient air; (*), (+), $P = 0.1$.

exposed to ambient air (mean 2002, 371 ± 10.9 , $F = 13.5$, $P = 0.002$, $n = 4$).

The vertical distribution of LAI at its maximum was measured at 10-cm intervals on 7 August 2002; elevated $[\text{CO}_2]$ caused a slight increase in LAI in the top layer of the canopy ($F = 7.91$, $P = 0.006$, $n = 4$) and a nonsignificant decrease in LAI at the bottom of the canopy ($F = 0.61$, $P = 0.44$, $n = 4$; Fig. 2a). These differences became more pronounced in September when LAI was declining (Fig. 2b). In 2001 we were unable to detect any effect of $[\text{CO}_2]$ on the profile of LAI. Elevated $[\text{CO}_2]$ had no consistent effect on canopy depth in either year (Fig. 3). Higher LAI, and the altered profile of LAI without a corresponding increase in canopy depth, suggest

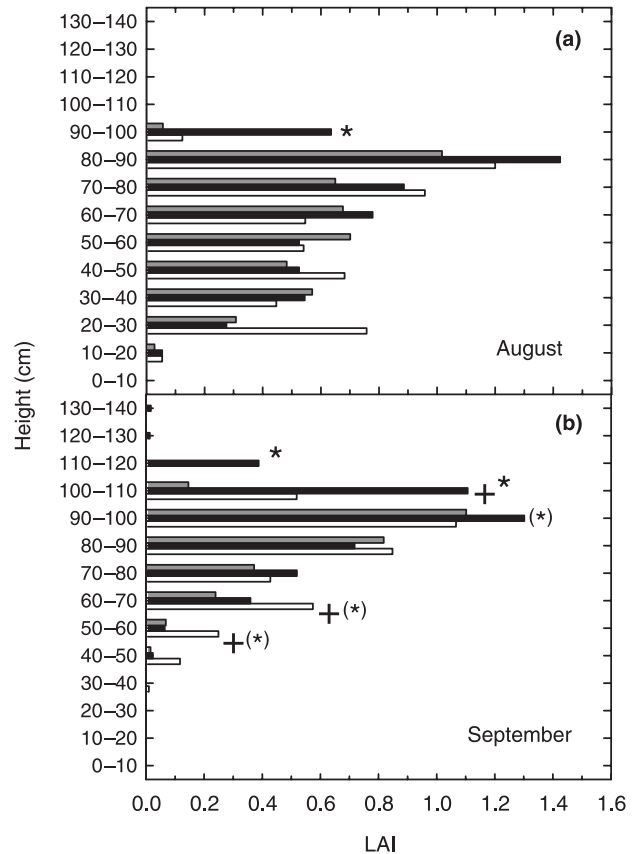


Fig. 2 Vertical distribution of leaf-area index (LAI) measured by harvesting soybean (*Glycine max*) plants at 10-cm intervals in 2002 from ground level to top of canopy. Ambient air, open bars; $550 \mu\text{mol mol}^{-1} \text{CO}_2$, closed bars; $1.2 \times$ ambient $[\text{O}_3]$, grey bars. (a) Vertical distribution at maximum LAI on 7 August 2002; (b) vertical distribution of LAI on 3 September 2002. Each bar represents least-squared mean ($n = 4$), SE not shown. *, +, Significant differences ($P = 0.05$) between elevated $[\text{CO}_2]$ or $[\text{O}_3]$ and ambient air; (*), (+), $P = 0.1$.

that (at least in 2002) elevated $[\text{CO}_2]$ led to a denser canopy that was shifted upwards relative to ambient air.

At the time of maximum LAI, we were unable to detect an effect of elevated $[\text{O}_3]$ on LAI at the bottom of the canopy ($F = 0.36$, $P = 0.55$, $n = 4$; Fig. 2a). However, elevated O_3 subsequently accelerated senescence (Fig. 1). From the time of maximum LAI onwards, canopy depth was lower in elevated $[\text{O}_3]$ relative to ambient air (on day 249, $F = 13.8$, $P = 0.0002$, $n = 4$). The negative effect of $[\text{O}_3]$ on canopy depth became more apparent as senescence progressed (Fig. 3b). Thus in elevated O_3 at maximum LAI the canopy was shorter, denser and shifted slightly upwards compared with ambient air. However, rapid senescence in elevated $[\text{O}_3]$ reduced LAI, and thus canopy density, towards the end of the growing season.

Leaf phenology and chemistry

No effects of elevated $[\text{CO}_2]$ on individual leaf duration in any cohort were detected (e.g. cohort 4, $F = 0.85$, $P = 0.39$,

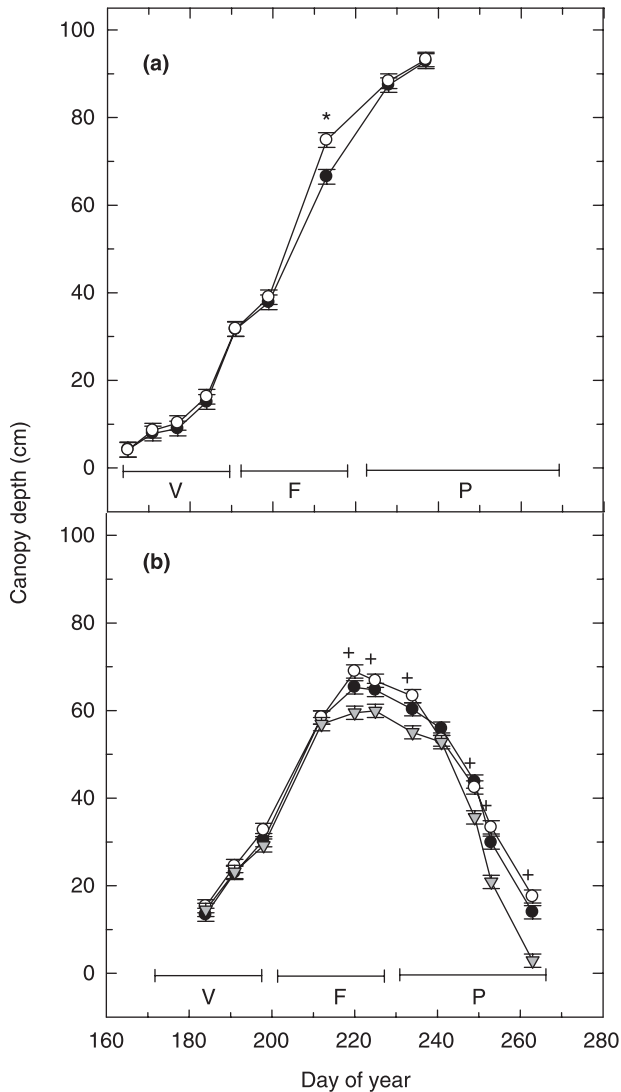


Fig. 3 Canopy depth (cm) of soybean (*Glycine max*) plants grown in ambient air (open circles); $550 \mu\text{mol mol}^{-1} \text{CO}_2$ (closed circles); $1.2 \times$ ambient O_3 (grey triangles). In 2001 only ambient and elevated $[\text{CO}_2]$ treatments were present (a); the elevated $[\text{O}_3]$ treatment was added in 2002 (b). Each point represents a least-squared mean \pm SE ($n = 4$) calculated from repeated measures ANOVA. Developmental stages of soybeans indicated at the bottom of each graph: V, vegetative; F, flowering; P, pod filling. *, +, Significant differences ($P = 0.05$) between elevated $[\text{CO}_2]$ or $[\text{O}_3]$ and ambient air; (*), (+), $P = 0.1$.

$n = 4$); however, across all cohorts there was a significant effect of CO_2 on leaf duration ($F = 3.6$, $P = 0.06$, $n = 4$). Earlier senescence of leaves exposed to elevated $[\text{O}_3]$ reduced the duration of cohorts 2, 3 and 6 by 4–7 d (Fig. 4). Because leaf duration was measured on a subset of leaves separate from those used for N determination, there was a discrepancy between the final date a leaf was sampled for N content and the date of leaf senescence (Fig. 4).

Elevated $[\text{CO}_2]$ significantly reduced SLA in cohorts 1 and 2. On day 172, the average SLA of leaves in cohort 2 grown

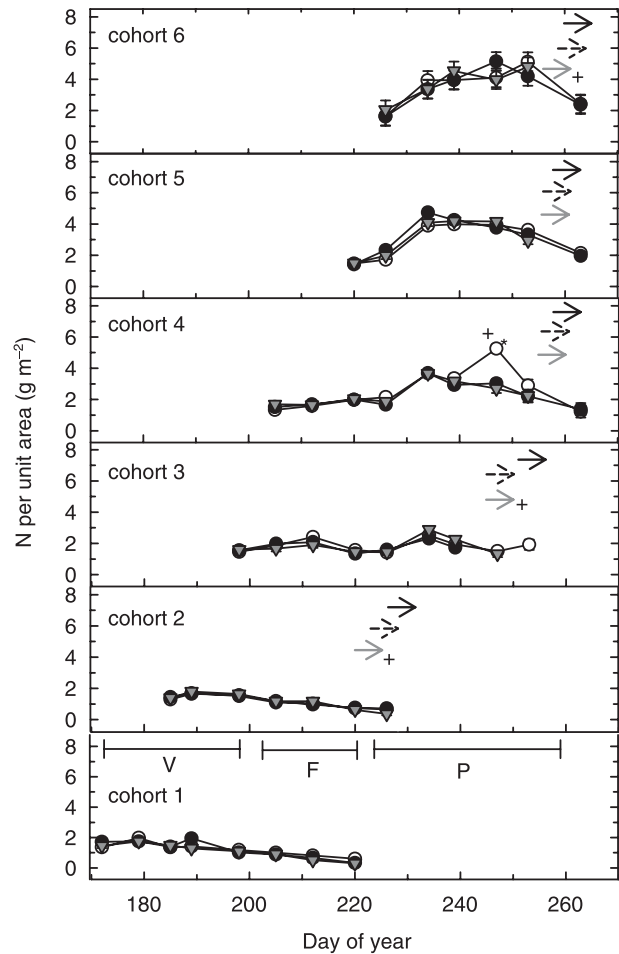


Fig. 4 Nitrogen content in individual leaves of each soybean (*Glycine max*) cohort throughout the 2002 growing season. Ambient air, open circles; $550 \mu\text{mol mol}^{-1} \text{CO}_2$, closed circles; $1.2 \times$ ambient O_3 , grey triangles. Cohorts 1–6 represent nodes 1, 3, 7, 10, 13 and 17, respectively. Developmental stages of soybeans indicated at top of cohort 1 panel: V, vegetative; F, flowering; P, pod filling. Date of leaf senescence, black (ambient); dashed (CO_2); grey (O_3) arrows (not recorded for cohort 1). End-point of arrow corresponds to date of senescence. Each data point represents least-squared means \pm SE ($n = 4$). Where error bars are not visible they are smaller than the points. *, +, Significant differences ($P = 0.05$) between elevated $[\text{CO}_2]$ or $[\text{O}_3]$ and ambient air; (*), (+), $P = 0.1$.

under ambient air and elevated $[\text{CO}_2]$ was 419 ± 15 and $319 \pm 16 \text{ cm}^2 \text{ g}^{-1}$, respectively, and these values declined to 286 ± 15 and $245 \pm 15 \text{ cm}^2 \text{ g}^{-1}$ by day 198. However, no consistent effect of elevated $[\text{CO}_2]$ was detected for the other cohorts (data not shown). Elevated $[\text{O}_3]$ had no consistent effect on SLA, although on day 212 average SLA was significantly greater in cohort 3 exposed to elevated $[\text{O}_3]$ ($311 \pm 15 \text{ cm}^2 \text{ g}^{-1}$) relative to ambient air ($249 \pm 16 \text{ cm}^2 \text{ g}^{-1}$). Across treatments, SLA was greatest for expanding leaves (approx. $300\text{--}400 \text{ cm}^2 \text{ g}^{-1}$) and declined with age to approx. $100\text{--}200 \text{ cm}^2 \text{ g}^{-1}$ before abscission.

Table 1 Photosynthetic parameters derived from the measured response of photosynthesis to variation in incident irradiance in soybean (*Glycine max*) leaves grown in the sun and shade, and in ambient air, 550 $\mu\text{mol mol}^{-1}$ CO_2 or 1.2 \times ambient $[\text{O}_3]$

Treatment	$\phi_{c,\text{max}}$	A_{sat}	R_d	Θ	LCP
2001 (sun leaves)					
Ambient air	0.060 (0.002)	28 (1)	3.2 (0.3)	0.8 (0.02)	20 (0.6)
Elevated CO_2	0.067 (0.002)*	33 (1)*	3.7 (0.3)	0.8 (0.02)	18 (0.6)*
2001 (shade leaves)					
Ambient air	0.064 (0.002)	23 (2)	0.6 (0.3)	0.8 (0.02)	19 (0.6)
Elevated CO_2	0.069 (0.002)(*)	25 (2)	0.9 (0.3)	0.8 (0.02)	17 (0.6)*
2002 (shade leaves)					
Ambient air	0.059 (0.002)	22 (2)	1.2 (0.3)	0.9 (0.03)	18 (0.3)
Elevated CO_2	0.067 (0.002)*	25 (2)	1.3 (0.3)	0.9 (0.03)	16 (0.3)*
Elevated O_3	0.064 (0.002)	18 (2)	1.2 (0.4)	0.9 (0.03)	19 (0.4)

Measurements were made in July 2001 and 2002. $\phi_{c,\text{max}}$ ($\mu\text{mol CO}_2 \mu\text{mol PFD}^{-1}$), Maximum apparent quantum yield, calculated independently as slope of strictly linear light-limited portion of photosynthetic light-response curve; A_{sat} ($\mu\text{mol m}^{-2} \text{s}^{-1}$), light-saturated rate of photosynthesis; R_d ($\mu\text{mol m}^{-2} \text{s}^{-1}$), rate of dark respiration; Θ , curvature of the light-response curve; LCP ($\mu\text{mol m}^{-2} \text{s}^{-1}$, PFD), light compensation point. All values are for photosynthesis measured at the $[\text{CO}_2]$ in which plants were grown. Least-squared means of four experimental plots are presented with SEM in parentheses.

, Significant differences ($P = 0.05$) between elevated $[\text{CO}_2]$ or $[\text{O}_3]$ and ambient air; (), $P = 0.1$.

When comparing across all cohorts over the season, no significant effect of elevated $[\text{CO}_2]$ was detected on leaf N content ($F = 0.83$, $P = 0.4$, $n = 4$). There was, however, a significant effect of cohort as illustrated by the dynamics of leaf N across the season ($F = 51.6$, $P = 0.001$, $n = 4$; Fig. 4). In cohorts 1 and 2, N content declined slightly from approx. 2 g m^{-2} on day 189 to 0.3 g m^{-2} on day 220 as leaves became shaded. However, N content of cohort 5 increased to approx. 5 g m^{-2} on day 247 from 1 g m^{-2} on day 205, then declined to 1 g m^{-2} before abscission (Fig. 4). This rapid decline in N content may indicate its remobilization from lower to upper canopy leaves or adjacent developing pods. Elevated $[\text{CO}_2]$ had no effect on this pattern, and there were no significant effects of elevated $[\text{CO}_2]$ or cohort on leaf carbon content ($F = 0.05$, $P = 0.83$, $n = 4$; $F = 1.31$, $P = 0.26$, $n = 4$, data not shown).

The dynamics of leaf N in elevated $[\text{O}_3]$ mirrored those in ambient air, and no significant effect of elevated $[\text{O}_3]$ was detected on leaf N content ($F = 1.08$, $P = 0.3$, $n = 4$). The only time elevated $[\text{O}_3]$ affected leaf N content, either across or within cohorts, was on day 247 in cohort 4, when leaf N content was approx. 5.3 ± 0.9 g m^{-2} in ambient air compared with 2.7 ± 0.9 g m^{-2} in elevated $[\text{O}_3]$. No significant effect was detected of elevated $[\text{O}_3]$ or cohort on leaf carbon content ($F = 1.4$, $P = 0.25$, $n = 4$; $F = 0.2$, $P = 0.66$, $n = 4$; data not shown).

Nitrogen was expressed per unit leaf area to avoid the confounding effects of age- or treatment-related changes in SLA. When expressed per unit leaf mass, changes in N content mirrored changes in SLA.

The maximum apparent quantum yield of photosynthesis ($\phi_{c,\text{max}}$) was greater in sun and shade leaves grown and measured in elevated $[\text{CO}_2]$ compared with ambient air (in 2002, $F = 11.0$, $P = 0.01$, $n = 4$; Table 1). The increase in $\phi_{c,\text{max}}$ under elevated $[\text{CO}_2]$ contributed to a decrease in the LCP of leaves in the upper and lower canopy (in 2002, $F = 33.0$,

$P = 0.001$, $n = 4$). The light-saturated rate of photosynthesis (A_{sat}) also was greater in leaves at the top of the canopy exposed to elevated $[\text{CO}_2]$ (Table 1). No effect of elevated $[\text{CO}_2]$ on A_{sat} , R_d and Θ was detected in leaves growing in the shade (in 2002, A_{sat} , $F = 2.4$, $P = 0.17$; R_d , $F = 0.02$, $P = 0.9$; Θ , $F = 0.3$, $P = 0.6$, $n = 4$; Table 1). Across treatments, A_{sat} and R_d were significantly greater in leaves at the top compared with the lower canopy. The LCP, Θ and $\phi_{c,\text{max}}$ were not different between leaves at the top and bottom of the canopy. Elevated $[\text{O}_3]$ had no detectable effect on any photosynthetic parameter.

Discussion

As initially hypothesized, elevated CO_2 caused an increase in $\phi_{c,\text{max}}$ and a decrease in LCP of shaded soybean leaves, and (at least in 2002) elevated CO_2 caused an increase in maximum LAI (Fig. 1). Following maximum LAI, canopy senescence was delayed in both genotypes under elevated $[\text{CO}_2]$. Contrary to our hypothesis, delayed senescence did not occur by the retention of shade leaves, but by continued addition of new sun leaves later into the growing season (Fig. 2). In contrast to the positive effects of elevated $[\text{CO}_2]$ on LAI, elevated $[\text{O}_3]$ when applied independently increased the rate of leaf senescence late in the season (Fig. 1), reducing LAD and the period for canopy carbon gain. This is the first study to examine the response of LAI in a legume crop to elevated CO_2 under FACE, and the results contrast with those from other crops grown under FACE (Drake *et al.*, 1997; Cowling & Field, 2003; Long *et al.*, 2004). Additionally, the magnitude of increase in maximum LAI was less than that reported by Ainsworth *et al.* (2002) from a meta-analysis of previous studies that relied on enclosures to administer treatments.

We hypothesized that higher $\phi_{c,\text{max}}$ and lower LCP would contribute to greater retention of leaves in the shade and thus

greater LAI in elevated $[\text{CO}_2]$ relative to ambient air. Based on a Beer Lambert approximation of light attenuation (Monsi & Saeki, 1953), shaded leaves in the canopy exposed to elevated CO_2 received approx. 7% less light in 2002 than those grown in ambient air. Elevated CO_2 caused an approx. 8–14% stimulation in $\phi_{c,\text{max}}$ and a 10–11% reduction in LCP (Table 1). Presumably, these changes in photosynthetic performance enhanced carbon gain for foliage deep in the canopy. While foliage at the bottom of the canopy experienced lower irradiance and improved photosynthesis, there was no indication that it was retained longer under elevated CO_2 (Figs 3,4). Thus the primary effect of improved photosynthesis under elevated CO_2 was to facilitate carbon gain in the shade (Singsaas *et al.*, 2000).

Leaf-area development can be limited by N availability (Hirose *et al.*, 1997), and this limitation is often cited as the reason for the absence of a detectable effect of elevated $[\text{CO}_2]$ on LAI (Arnone & Körner, 1995; Hartz-Rubin & DeLucia, 2001; Franklin & Ågren, 2002). When N is limiting, remobilization from older leaves to the upper canopy may accelerate their senescence and constrain the development of greater LAI under elevated $[\text{CO}_2]$ (Long, 1991; Hirose *et al.*, 1997). Because there was no detectable increase in maximum LAI in 2001, albeit with a different cultivar, leaf tissue was analyzed in 2002 to determine if elevated $[\text{CO}_2]$ affected the remobilization of N. As leaves in cohorts 1–4 aged and became shaded, their N concentrations declined (Fig. 4), which may indicate remobilization to the upper leaves. However, we were unable to detect increased remobilization of N from senescing leaves in soybeans grown in elevated $[\text{CO}_2]$. In cohorts 5 and 6, which were formed at the top of the canopy after maximum LAI, leaf N continued to increase while pods were developing (Fig. 4). The increase in leaf N content while demand for this nutrient by developing pods was presumably substantial suggests that, at least in 2002, the canopy was not limited by N availability. Nitrogen limitation also seems unlikely given that soybean is an N-fixer and maximum leaf N content reached 5% in 2002.

Increases in water-use efficiency and carbohydrate levels may also contribute to greater leaf size and LAI under elevated $[\text{CO}_2]$ (Pritchard *et al.*, 1999). Lower stomatal conductance and higher levels of foliar carbohydrates were measured at SoyFACE (Rogers *et al.*, 2004; Bernacchi *et al.*, 2005). As part of a separate investigation of the impacts of herbivory on LAI, in 2002 elevated $[\text{CO}_2]$ significantly increased leaf size in July (ambient $[\text{CO}_2]$ mean leaf area, $86 \pm 1 \text{ cm}^2$; elevated, $89 \pm 1 \text{ cm}^2$) and August (ambient $[\text{CO}_2]$ mean leaf area, $130 \pm 1 \text{ cm}^2$; elevated, $155 \pm 1 \text{ cm}^2$). In poplars exposed to elevated $[\text{CO}_2]$, increased leaf size contributed to higher LAI but, unlike in our study, this effect diminished after canopy closure (Gielen *et al.*, 2001; Taylor *et al.*, 2003; Tricker *et al.*, 2004).

Elevated $[\text{CO}_2]$ may increase rates of canopy development by increasing the rate of leaf expansion (Pritchard *et al.*, 1999). This probably occurred in other studies where the initial

growth in LAI was accelerated, but no response of maximum LAI to elevated $[\text{CO}_2]$ was detected (Arnone & Körner, 1995; Hirose *et al.*, 1996; Hartz-Rubin & DeLucia, 2001; Hymus *et al.*, 2002). We did not detect an increase in the rate of canopy development of soybeans grown in elevated $[\text{CO}_2]$, but there was an extended period of canopy development.

Ultimately, we cannot completely separate the effects of genotype and climate; however, differences between years in the response of maximum LAI to elevated $[\text{CO}_2]$ were probably related to the genotypes planted rather than variations in weather. Pana, the genotype planted in 2001, reached heights of approx. 123 cm, while the maximum plant height of the Pioneer genotype in 2002 was only 90 cm (data not shown). In 2001 there were no differences in the pattern of biomass allocation between stems and leaves in elevated $[\text{CO}_2]$; however, in 2002 the proportion of biomass allocated to leaves was significantly greater than that allocated to stems (Morgan *et al.*, 2005a). The weather in 2001 and 2002 was similar, with approximately the same amount of precipitation (2001, 286 mm; 2002, 324 mm) and mean temperature (2001, 23°C ; 2002, 24°C) during the growing season. Thus it is most likely that the flexibility in above-ground allocation displayed by the Pioneer genotype enabled it to increase maximum LAI under elevated $[\text{CO}_2]$ in 2002.

In addition to the stimulation of maximum LAI, elevated $[\text{CO}_2]$ may indirectly enhance LAD and seasonal carbon gain by delaying the rate of canopy senescence. Changes in phenology are often attributed to variation in temperature (Menzel & Fabian, 1999; Penuelas *et al.*, 2002; Root *et al.*, 2003); however, elevated $[\text{CO}_2]$ also can affect phenology (Li *et al.*, 2000; Jach *et al.*, 2001; Rogers *et al.*, 2004; D. Karnosky, S. Long, G. Taylor and co-workers, unpublished data). The LAI of soybeans grown in elevated $[\text{CO}_2]$ was approx. 54 and 33% greater than in ambient air at the end of the 2001 and 2002 seasons, respectively (Fig. 1). Measurements of the profile of soybean LAI over the 2002 season revealed that, after maximum LAI was attained, senescence of the whole canopy was delayed in elevated compared with ambient $[\text{CO}_2]$ by the addition of new leaves at the top of the canopy (Fig. 2).

Although high relative to other species, exposure to elevated $[\text{CO}_2]$ further increased the LAI and LAD of soybean. The mechanisms contributing to this stimulation were, however, more complex than initially hypothesized. Larger leaves under elevated $[\text{CO}_2]$ contributed to the greater LAI and canopy density. It is possible that increased $\phi_{c,\text{max}}$ and decreased LCP of shade leaves contributed to increased carbon gain at the bottom of the canopy up to the time of maximum LAI. However, the leaf area profile (Fig. 2) indicated that there was less leaf area at the bottom of the canopy in elevated $[\text{CO}_2]$, suggesting that canopy senescence was delayed not by the retention of shade leaves, but by the growth of new leaves at the top of the canopy.

In contrast to elevated $[\text{CO}_2]$, exposure to $1.2 \times$ current ambient $[\text{O}_3]$ accelerated senescence in the soybean canopy

(Fig. 1), which reduced LAD. There was no effect of elevated $[O_3]$ on maximum LAI (Fig. 1) or photosynthesis measured in July (Table 1). Morgan *et al.* (2004) was also unable to detect an effect of elevated $[O_3]$ on instantaneous photosynthesis of soybean leaves early in the season. However, elevated $[O_3]$ significantly reduced A_{sat} on leaves that developed late in the season. The inhibition of photosynthesis late in the season, and the acceleration of canopy senescence, suggest that damage increases with cumulative O_3 dose. Decreased foliar retention was also measured in the only other FACE system with an elevated $[O_3]$ treatment (Karnosky *et al.*, 2003). The average seasonal level of $[O_3]$ in this experiment of 20% above ambient is at the lower level of scenarios predicted by the Intergovernmental Panel on Climate Change for 2050 (Prather *et al.*, 2001). Therefore future reductions in LAI caused by elevated $[O_3]$ may be greater than observed in this experiment.

The combination of higher rates of photosynthesis (Bernacchi *et al.*, 2005) and greater LAD contributed to higher soybean productivity in elevated $[CO_2]$ (Morgan *et al.*, 2005a). However, accelerated canopy senescence contributed to lower seed yield in elevated $[O_3]$ (Morgan *et al.*, 2005b). Although, bean yield increased by approx. 15% in elevated $[CO_2]$, the harvest index declined by 3%, which represents a decline in partitioning to seed dry mass. Elevated $[O_3]$ reduced seed yield by about 15% (Morgan *et al.*, 2005b) and, as for LAI, the effects on above-ground biomass were visible towards the end of the season. In elevated $[CO_2]$ increases in seed yield were driven by both higher rates of leaf-level photosynthesis and increases in both duration and size of the canopy (Fig. 1; Morgan *et al.*, 2005a). In elevated $[O_3]$, however, losses in seed yield were driven primarily by declines in LAI and canopy duration (Fig. 1; Morgan *et al.*, 2005b). It remains to be seen if, as in other systems, elevated $[O_3]$ will offset the positive effects of CO_2 on soybean when the two gases are elevated simultaneously (Karnosky *et al.*, 2003).

In addition to the economic implications, the contrasting effects of elevated $[CO_2]$ or $[O_3]$ on canopy structure and duration for the soybean agro-ecosystem that occupies approx. 30 million ha (USDA, 2001) may have an impact on regional energy and water cycles (Betts *et al.*, 1997; Woodward *et al.*, 1998; Raddatz, 2003; Zavaleta *et al.*, 2003). Although many varieties of soybean are planted in the US mid-west, the current study was limited to examining the response of two varieties at a single site. Despite this limitation, by incorporating the results of this research into regional circulation models, it may be possible to resolve how structural changes in the canopy in elevated $[CO_2]$ or $[O_3]$ could affect soybean productivity and simultaneously feed back to atmospheric energy and water cycles over the mid-western USA.

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