Soybean photosynthesis and yield with global change

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Global warming can negate the expected CO$_2$ stimulation in photosynthesis and productivity for soybean grown in the Midwest United States

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Abstract

Extensive evidence shows that increasing CO₂ concentration ([CO₂]) stimulates, and increasing temperature decreases, both net photosynthetic carbon assimilation (A) and biomass production for C₃ plants. However the [CO₂]-induced stimulation in A is projected to increase further with higher temperature. While the influence of increasing temperature and [CO₂], independent of each other, on A and biomass production have been widely investigated, the interaction between these two major global changes have not been tested on field-grown crops. Here, the interactive effect of both elevated [CO₂] (~585 μmol mol⁻¹) and temperature (+3.5 °C) on soybean (Glycine max Merr.) A, biomass, and yield were tested over two growing seasons in the Temperature by Free-Air CO₂ Enrichment experiment at the Soybean Free Air CO₂ Enrichment facility. Measurements of A, stomatal conductance (gₛ), and intercellular [CO₂] (Cᵢ) were collected along with meteorological, water potential, and growth data. Elevated temperatures caused lower A, which was largely attributed to declines in gₛ and Cᵢ and led in turn to lower yields. Increasing both [CO₂] and temperature stimulated A relative to elevated [CO₂] alone on only two sampling days during 2009 and on no days in 2011. In 2011, the warmer of the two years, there were no observed increases in yield in the elevated temperature plots regardless of whether [CO₂] was elevated. All treatments lowered the harvest index for soybean, although the effect of elevated [CO₂] in 2011 was not statistically significant. These results provide a better understanding of the physiological responses of soybean to future climate change conditions and suggest that the potential is limited for elevated [CO₂] to mitigate the influence of rising temperatures on photosynthesis, growth, and yields of C₃ crops.
Introduction

The global atmospheric concentration of CO₂ ([CO₂]) is predicted to rise from current concentrations of ~390 μmol mol⁻¹ to between 730 and 1020 μmol mol⁻¹ by 2100 (IPCC, 2007; Canadell et al., 2007). An increase of [CO₂] is expected to stimulate photosynthesis, as has been demonstrated experimentally for a wide range of C₃ species (e.g., Curtis & Wang, 1998; Bernacchi et al., 2003a; Nowak et al., 2004; Long et al., 2004; Ainsworth & Long, 2005; Ainsworth & Rogers, 2007). Increasing [CO₂] coupled with the continued accumulation of other greenhouse gases (GHG) in the atmosphere is predicted to increase the global average temperatures between 2.4 to 6.4°C by the end of the century (A1FI scenario; IPCC, 2007). While the thermal optimum \( (T_{opt}) \) for photosynthesis ranges between 20°C to 35°C for C₃ species (Sage et al., 2008), plants have considerable capacity to acclimate to long-term temperature increases under current [CO₂]. Consequently, the combined changes in the atmospheric composition and climate are likely to impart significant effects on terrestrial ecosystems because both CO₂ and temperature are critical determinants of photosynthetic rates (Sage & Kubien, 2007).

Soybean, grown in rotation with maize, represents the largest land-use in the United States combining to cover an estimated area of 67 million hectares (USDA, 2011). Soybean is the fourth most important commodity crop globally with nearly 40% of world production coming from the Midwestern US region. As one of the two crops that dominates the Midwestern landscape, soybean strongly impacts regional ecosystem services, such as water quality, hydrologic cycling and, as a grain legume, soil nitrogen production. Thus, global change-induced alterations in soybean production may have large-scale socioeconomic and ecological impacts.

Effective strategies to adapt agricultural production to global change require improved predictions of crop responses to global change scenarios. The Soybean Free Air CO₂ Enrichment (SoyFACE) research facility was developed to address the molecular, physiological, and growth responses of the Midwestern crops soybean and maize to global change through the use of the Free Air CO₂ Enrichment (FACE) technology (Miglietta et al., 2001). This technology provides in-field fumigation of CO₂ to simulate future atmospheric conditions while growing crops under otherwise natural field conditions using current agronomic practices. Previous results from SoyFACE showed that the daily integral of carbon uptake (\( A' \)) is increased by ~25% for soybean grown under elevated [CO₂] (Rogers et al., 2004; Bernacchi et al., 2006). This occurs despite the accompanying photosynthetic acclimation most evident as the reduction in the activity and/or
content of the primary carboxylating enzyme Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) (Bernacchi et al., 2005; Ainsworth & Long, 2005). However, in soybean and other C3 grain crops, CO2-induced increases in growth and yield are observed to be lower than the increase in net photosynthesis (Morgan et al., 2005; Long et al., 2006). In addition to increasing carbon uptake (A) of soybean, elevated [CO2] also reduces stomatal conductance (gs) relative to plants grown in ambient [CO2] (Ainsworth et al., 2002; Rogers et al., 2004; Bernacchi et al., 2006; Bernacchi et al., 2007). This reduction in gs can lead to lower canopy water use, improved water use efficiency and conservation of soil moisture (Bernacchi et al., 2007; Leakey et al., 2006).

While FACE experiments have improved our understanding of the plant physiological responses to a [CO2] enriched atmosphere, the interactive effects of the two major global change factors, rising [CO2] and warming have not been investigated by a direct manipulative experiment under field conditions for any crop. It has been shown that the stimulatory effect of elevated [CO2] on A is enhanced at higher daily temperatures (Bernacchi et al., 2006), in agreement with theory (e.g., Long 1991). However, this analysis was based on the natural variation in temperature during 15 days over three growing seasons thus confounding the correlation with other environmental factors and neglecting long-term acclimation effects to higher temperature (Long 1991; Sage & Kubien, 2007). Recent multiple regression analyses of historical yield data and growing season temperatures indicate that yields of soybean are depressed in warmer years (Lobell & Field, 2007; Kucharik & Serbin, 2008), with a steeper decline in yield above, compared with the incline below, a critical temperature (Schlenker & Roberts, 2009). It is also predicted that a 0.8°C increase in temperature could increase soybean yields in the Midwestern US by ~1.7% based on the mean air temperatures of 22.5°C but decrease yields for the warmer conditions in the Southern US (Hatfield et al., 2011). The results from this review (Hatfield et al., 2011) suggest that the impact of warming on yield of soybean is highly dependent on baseline conditions. Because of the empirical nature of these analyses, the mechanisms involved in the decline of soybean productivity above the thermal optimum are unclear (Ainsworth & Ort, 2010). Accurately projecting the impact of global change on crop productivity relies on understanding how rising [CO2] and increasing temperature together influence photosynthesis, growth, phenology, and yield of major crop species.
The objectives of this study were to quantify and understand the biological processes involved in photosynthesis, growth and biomass production for soybean under a warmer and CO₂-enriched atmosphere. Specifically, we hypothesized that increasing the growing season temperature of a soybean canopy by 3.5°C above the current ambient conditions, an increase expected midway through this century for terrestrial areas (Rowlands et al., 2012), will result in lower photosynthesis, biomass productivity and yields. This is consistent to what has been observed historically for warmer years. We further hypothesized that the combination of elevated [CO₂] and warmer temperatures will yield higher photosynthesis, biomass accumulation and yield relative to the elevated [CO₂] treatment alone. Given the high interannual variability in climate, these hypotheses were tested over two growing seasons; we hypothesized that the responses to elevated [CO₂], warmer temperature, and the combined treatment would be consistent across growing seasons.

Results

Meteorological conditions contrasted between the 2009 and 2011 growing seasons

The mean temperatures for July, August and September were lower in 2009 and higher in 2011 when compared to the 30-year mean (Table 1). The largest departure from normal in 2009 was observed in July with temperatures approximately 2.9°C below average. In 2011, July had the highest departure with mean temperatures of 2.8°C above average. In both years, April and May experienced higher than average precipitation ensuring that both growing seasons began with soil moisture near field capacity. The entire 2009 growing season experienced near-average precipitation, whereas in 2011 there were significant shortfalls of 60% in July and 47% in August compared to the 30-year means for those months (Table 1). Growth stages (Supplementary Table 1) and specific meteorological conditions (Fig. 1; Supplementary Table 2) associated with each measurement day varied within and between growing seasons.

Photosynthesis and stomatal conductance varied among CO₂, temperature, and combined treatments.

Measurements of A were collected throughout the daylight hours on seven (2009) and six (2011) days throughout each growing season. The measurements were taken in conditions set to mimic the field conditions at the specific time (Supplementary Figs. 1 and 2).
measurements showed variable responses of the key gas exchange parameters $A$, $g_s$, $C_i$, and iWUE over the course of the two growing seasons (Supplementary Tables 3 and 4). Relative to the control, the eC plots yielded higher $A$, the eT plots lower $A$ and the eT+eC plots higher $A$ over most of the time points (Supplementary Figs. 1 and 2).

The diurnal measurements of $A$ were integrated across the daylight hours to obtain the daily integral of photosynthesis ($A'$). Overall, the effect on $A'$ of eT and of eC relative to the control plots were consistent across both growing seasons, although the increase in eC and the decrease in eT was greater in 2011 (Fig. 2). In 2009 there was a statistically significant Temperature by CO$_2$ interaction. This interaction is explained by a lack of statistical difference between the control and eT plot while the eT+eC plot had much higher $A'$ relative to the control (Tables 2 and 3). In 2009 both eC and eT+eC treatments had significantly higher $A'$ than the ambient [CO$_2$] treatments (i.e., control and eT; Fig. 2; Table 2), although eC and eC+eT were not detectably different from each other (Table 3). The responses of $A'$ among the treatments in 2009 did not result in statistically significant interactions of main effects with DOY (Table 2; Supplementary Table 3).

In contrast to 2009, in 2011 there were no statistically significant interactive effects of temperature and [CO$_2$] on $A'$ suggesting that the main effects alone were responsible for the observed differences. Elevated [CO$_2$] increased $A'$ within a temperature treatment (eC vs. control and eT+eC vs. eT) and elevated temperature decreased $A'$ within a CO$_2$ treatment (eT vs. control and eT+eC vs. eC; Tables 2 and 3; Fig. 2). The CO$_2$ effect, based on percentage change, was greater than the temperature effect, thus the combined influence of increasing temperature and rising [CO$_2$] stimulated $A'$ in the eC+eT treatment relative to the control, but the increase was lower than the eC vs. control comparison (Table 3, Fig. 2). This response was different from the pattern observed in 2009 where $A'$ in eT+eC showed the highest value although significantly different from eC. The effect of temperature varied by DOY in 2011 when temperature did not have significant effects on $A'$ on two measurement dates (DOY 186 and 251; Fig. 2; Supplementary Table 4).

Over both years, elevated [CO$_2$] and warming reduced $g_s$ when treatments were applied independently and in combination (Fig. 2; Table 2), with the eT+eC treatment showing the lowest $g_s$ overall for both years (Table 3; Fig. 2). The influence of CO$_2$ relative to temperature, however, appears to change from 2009 to 2011. In 2009, elevated [CO$_2$] has a greater impact on
$g_s$, compared with temperature whereas in 2011 temperature has a bigger impact on $g_s$ than [CO2]
(Fig. 2). The impact of elevated temperature and/or [CO2] on $g_s$ varied based on DOY for both
years (Table 2; Supplementary Tables 3 and 4). The [CO2] treatment differences were
statistically significant on all but one day for each of the years. The temperature-induced
reduction in $g_s$ was statistically significant on four of the seven measurement days for 2009 and
on all measurement days in 2011 (Fig. 2; Table 3; Supplementary Tables 3 and 4).

Over both growing seasons, elevated temperatures reduced and elevated [CO2] increased
$Ci$ relative to the controls (Tables 2 and 3; Fig. 2). While the pattern of responses were similar
over both growing seasons, the influence of temperature in 2011 was much greater than that
observed in 2009. Despite a significant temperature by CO2 interaction in 2011, the increase in
$Ci$ for the eC treatment and the decrease in $Ci$ for the eT treatment appeared to have an additive
effect on the eT+eC treatment for both years.

The percent change in $A'$ within a [CO2] level (eT vs. Control and eT+eC vs. eC) was
plotted as a function of daily maximum canopy temperature based on the temperatures measured
in the heated plots (Fig. 3). The percent change in $A'$ between the eT+eC and eC treatments
increased with temperature in 2009 and decreased with temperature in 2011 (Fig. 3). The decline
in $A'$ for eT+eC relative to eC in 2011 was rapid, and stabilized at daily maximum temperatures
above 34°C. The eT treatment decreased $A'$ slightly relative to control in 2009 and remained
about 20% lower for all daily temperatures in 2011 (Fig. 3).

The relationship of instantaneous $A$ to $g_s$ increased to an asymptote for all treatments (Fig.
4). While it was difficult to discern differences within a [CO2] treatment (eT vs. Control and
eT+eC vs. eC), it was clear that for a given $g_s$ the elevated [CO2] treatments had much higher $A$.
Averaging $A$ and $g_s$ within each measurement day for both years to calculate the daily mean and
seasonal mean intrinsic water use efficiency (iWUE; $A/g_s$) indicated significant differences
among the treatments (Table 2). For both years, there were statistically significant increases in
iWUE associated with elevated temperature and with [CO2] treatment (Table 3; Fig. 4). While
the responses of iWUE were similar for both growing seasons, the CO2 effect was greater in
2009 than in 2011 and the temperature effect was greater in 2011 than in 2009 (Table 3; Fig. 4).
For both years it appears that the influence of the two main effects was additive (Table 3; Fig. 4).
Elevated temperature slightly reduced leaf water potential and increased leaf-to-air VPD.

Leaf water potential estimated using thermocouple psychrometry was determined on leaf tissue collected at midday of each measurement date. Temperature, regardless of the [CO2], had a small but significant effect on total water potential (WP) and turgor pressure (TP) in both years (Fig. 5; Table 2). Elevated temperature decreased growing season mean WP by 7% in 2009 and by 16% in 2011 (Table 3). While no statistically significant results were observed with osmotic potential (OP) for either year; the temperature main effect decreased TP by 17% in 2009 and 16% in 2011. The seasonal and daily mean values of WP, OP, and TP in 2011 were nearly half the values obtained in 2009 (Fig. 5).

The seasonal mean leaf-to-air vapor pressure deficit (VPD) obtained from the gas exchange system during the in situ measurements showed higher values in the warmer (1.58 ± 0.022 kPa for eT, 1.77 ± 0.023 kPa for eT+eC) relative to the reference (1.27 ± 0.018 kPa for control, 1.49 ± 0.020 kPa for eC) temperature plots in 2009. The relative responses in 2011 were similar to 2009 with the heated plots showing higher VPD (1.93 ± 0.030 kPa for eT, 2.09 ± 0.033 kPa for eT+eC) relative to the reference (1.51 ± 0.025 kPa for control, 1.68 ± 0.026 kPa for eC) temperature plots. These values gave percentages deviations from control of 24.7% for eT, 17.6% for eC and 39.6% for eT+eC in 2009; and 28.1% for eT, 11.4% for eC and 38.4% in 2011.

Elevated CO2 increased biomass and yields but the impact of temperature varied with growing season.

Both growing seasons resulted in statistically significant CO2 by temperature interactions for above-ground biomass (AGB) indicating that a synergistic effect occurred between these two global changes. The treatment responses, however, were not consistent over both growing seasons. In 2009 the only two differences among the treatment were between the eT+eC comparison with the control plot and the eT+eC comparison with the eT plot (Tables 2 and 3; Fig. 6). In 2011 AGB in the eC plots were higher than the control and the eT+eC plots (Tables 2 and 3; Fig. 6).

The general responses of seed yield (SY) for both growing seasons were relatively similar to the observations of AGB, however, in 2009 the only statistically significant differences occurred between the eT+eC and the eT treatment, with the eT treatment showing the lowest and the eT+eC treatment the highest SY (Tables 2 and 3; Fig. 6). The impact of temperature had a
strong negative impact on yields in 2011 with the eT and the eT+eC plots having lower SY compared with the both the control and the eC plots (Tables 2 and 3; Fig. 6).

All treatments appeared to decrease the harvest index (HI) in both years (Fig. 6) however in 2011 the only statistically significant differences were between the eC+eT and the control (Table 2). Overall, the HI in 2011 was much lower relative to 2009 and the response of HI to the various treatments was amplified. In 2011 neither eT nor eC differed from the control plots but the eC+eT treatment differed from the control, eT, and eC plots (Table 2). The differences between the treatments and the control in 2011 were greater than in 2009, however there was also much greater variance in the measurements (Fig. 6).

Discussion:

This experiment was designed to test the hypotheses that (1) increasing soybean canopy temperature above ambient will result in lower photosynthesis, biomass productivity and yields, an effect that is historically characteristic of warmer years; and (2) that concomitant increases in [CO₂] and temperature will result in higher photosynthesis, biomass production and yields compared to elevated [CO₂] alone. The results only partially support the first hypothesis. Significant differences between the control and eT treatment were observed only for photosynthesis (Fig. 2) and SY (Fig. 6) in 2011, the warmer of the two growing seasons. The results also indicate that the second hypothesis is not supported. In 2009, the eT+eC treatment yielded significantly greater A on only two sampling days relative to the eC treatment and in 2011 the eT+eC treatment yielded significantly lower photosynthesis than the eC treatment. Moreover, in 2011 the response of photosynthesis for the eT+eC treatment, which was slightly higher than the control plots, did not translate to increases in SY, rather the eT+eC plot had lower SY than any other treatment. The combined eT+eC treatment yielded higher photosynthetic rates in both years compared with the eT treatment, suggesting that eC is able to at least partially mitigate the negative effects of eT, but this response did not lead to higher SY for the eT+eC plot compared with the eT plot in 2011. In 2011 the eT plots, with (eT+eC) or without (eT) elevated [CO₂] yielded lower SY relative to the control.

The influence of rising [CO₂] on photosynthesis, growth and yield has been well documented for soybean (Rogers et al., 2004; Bernacchi et al., 2006) as well as for other species (Bernacchi et al., 2003a; Nowak et al., 2004; Long et al., 2004; Ainsworth & Long, 2005;
Ainsworth & Rogers, 2007). Consistent with previous research on soybean grown at SoyFACE (e.g., Leakey et al., 2009), significant increases in photosynthesis in elevated [CO₂] were observed in both years (Tables 2 and 3). The 2009 growing season was the only time out of ten growing seasons at SoyFACE in which increases in SY with elevated [CO₂] were absent. One of the key benefits of an increase in photosynthesis under elevated [CO₂] is the reduction of photorespiration (Long, 1991; Long et al., 2004; Ainsworth & Rogers, 2007). The smaller than predicted response in 2009 could be attributed to cooler temperatures where the inhibition of photosynthesis due to photorespiration was low (Jiao & Grodzinski, 1996). This, coupled with a large decrease in gs (Fig. 2) under elevated [CO₂] could have contributed to the muted yield response in 2009. The 2011 growing season was warmer than typical, thus the ability to suppress photorespiration at high [CO₂] likely conferred a greater benefit under these conditions. The 2011 growing season showed higher biomass and yields for the elevated [CO₂] grown plants as observed for most growing seasons at SoyFACE.

The heated plots showed consistently reduced photosynthetic carbon uptake for both growing seasons relative to the control. It was clear that the increase in temperature had a greater effect on reducing photosynthesis in 2011 than in 2009, likely driven by the much warmer background temperatures in 2011. This is observed in the seasonal percent deviation of A’ for eT vs. Control, which shows a slight decrease in 2009 and a much larger decrease in 2011 (Table 3). This is also seen in the percent deviation of A’ versus daily maximum temperature for eT vs. Control (Fig. 3). In 2009 as temperature in the heated plots increased, the percent difference of A’ between the eT+eC vs. eC plots continued to increase. Contrary to this, the differences between the eT vs. control plots declined, indicating greater heat-induced suppression of A’ with temperature. In 2011 A’ was consistently lower in the eT treatment relative to the control by ~20% regardless of daily maximum temperature whereas the stimulation associated with the eT+eC plot dropped as temperatures rose (Fig. 3). These results suggest that in 2009 photosynthesis in the non-heated plots were below the thermal optimum driving photosynthesis higher as heat was applied whereas in 2011 photosynthesis for the non-heated plots was operating above the thermal optimum such that any further increases in temperatures drove photosynthesis down.

The potential importance of the interaction of elevated [CO₂] and increased temperature on photosynthesis is well described using mechanistic theory (e.g., Long 1991; Sage & Kubien
and demonstrated using correlative data collected over many days over different growing seasons (e.g., Bernacchi et al., 2006). The results presented here show that under field conditions, the importance of the elevated [CO₂] and temperature effect on photosynthesis, growth and yield is dependent on the extent of heating, on whether the temperature was above or below optimum at any particular time, and on the interaction with other environmental factors. Of particular importance is the role of changes associated with the underlying biochemistry of photosynthesis. Elevated [CO₂] is shown to down-regulate the maximum velocity for carboxylation (V_{c,max}) for soybean grown in elevated [CO₂], however this acclimation had little impact on photosynthetic rates as soybean is not V_{c,max} limited in elevated [CO₂] (Bernacchi et al., 2005). Acclimation has been shown to occur in response to higher growth temperature for V_{c,max} and maximum rate of electron transport (J_{max}; June et al., 2004; Onoda et al., 2005; Kattge and Knorr, 2007). Over the same growing seasons and coupled with the measurements presented here, in depth analysis of V_{c,max} and J_{max} for soybean at SoyFACE (Rosenthal et al., submitted) shows elevated [CO₂]-induced down-regulation of V_{c,max} consistent with previous reports on soybean but not for J_{max}. The elevated temperature plots show declines in J_{max}, regardless of whether [CO₂] is increased (Rosenthal et al., submitted).

To determine whether photosynthesis was predominately Rubisco- or RuBP-regeneration limited over each diurnal for all treatments we coupled the results from Rosenthal et al. (submitted) for V_{c,max} and J_{max} with the leaf photosynthesis model (Farquhar et al., 1980) corrected for temperature (Bernacchi et al., 2001; 2003b). Using this modeled data, we determined whether photosynthesis is Rubisco- or RuBP regeneration-limited for all data points collected over the two growing seasons. This analysis showed that over 85% of all data points were RuBP-limited (Supplemental Figs. 1 and 2). Because Rubisco-limited photosynthesis was rare, any down-regulation in V_{c,max} is likely to not influence A. Given its importance for RuBP regeneration limited A, the down-regulation of J_{max} is likely to drive down productivity.

The responses of photosynthesis to elevated [CO₂] and warmer temperature over these two growing seasons are not likely driven exclusively by the temperature and CO₂ sensitivity of Rubisco kinetics and RuBP regeneration (e.g., Rosenthal et al., submitted). Previous research at SoyFACE showed that elevated [CO₂] resulted in ~16% mean reduction in g_s (Bernacchi et al., 2006), which is substantially less than the reduction observed here (Table 3). Thus, the pronounced reduction of g_s under elevated [CO₂] and/or temperature over both years is likely to...
influence the deviation of the observations from theoretical responses. Over two sampling dates in 2009 (DOY 183 and 238) the differences in $g_s$ were minimal for the eT+eC vs. eC treatments (Fig. 2). The combined increase in [CO$_2$] and temperature for these two days yielded much higher $A'$, consistent with the theory presented previously (e.g., Long, 1991). All other measurements days during 2009 yielded $g_s$ values that were lower in the eT+eC relative to the eC treatment and did not show differences in $A'$ between these treatments. The temperature-induced reduction in $g_s$ led to lower $C_i$ within a CO$_2$ treatment (Figure 2). The effect of temperature resulted in a much greater decrease for $C_i$ in 2011 than in 2009. In 2011, $C_i$ in the eT+eC treatment was only ca 20% higher than the control despite atmospheric CO$_2$ being ca 50% higher.

Despite the reductions in $g_s$, the eT, eC and eT+eC treatments showed a higher iWUE (Fig. 4; Table 3). This increase in iWUE is predicted for plants grown in elevated [CO$_2$] as stomatal limitation to photosynthesis is consistently shown to be lower despite a decrease in $g_s$ (e.g., Bernacchi et al., 2005; Rosenthal et al., submitted). The elevated-temperature-grown plants also showed an increase in iWUE across both growing seasons relative to the control. The increase in iWUE for the heated treatments within a [CO$_2$] (eT vs. control and eT+eC vs. eC) was less than that measured within a temperature treatment (eC vs. control and eT+eC vs. eC). The increase associated with the eT treatment relative to the control occurred as a result of the decrease in $g_s$ being proportionately greater than the decrease in $A'$ (Table 3). This indicates that while all treatments experienced a higher iWUE, the mechanisms behind these responses varied among treatments.

A characteristic of an experiment that heats the canopy instead of the air will lead to an increase in VPD at the leaf surface, which likely leads to an increase in water use (Kimball, 2005; Kimball, 2011; De Boeck et al., 2012). Averaged across time points in which gas exchange measurements were collected, the effect of heating increased the leaf to air vapor pressure deficit by 0.3 kPa in 2009 and 0.4 kPa in 2011 within a CO$_2$ treatment (eT vs. control and eT+eC vs. eC). Both growing seasons experienced significant precipitation during spring, and 2009 had ample precipitation throughout the season (Table 1). Leaf water potential data for both years showed the same responses to temperature, with a general response of temperature, within a CO$_2$ treatment showing more negative WP relative to the non-heated reference plots (Fig. 5; Table 2). The differences in WP, OP and TP in 2011 compared to 2009 indicate that the
variability in climate between the two growing seasons had a dominant influence on leaf water potential relative to the treatment imposed by the infrared heating arrays. Moreover, the pairwise comparison analysis showed no statistical differences among treatments for the WP and TP variables in either year, suggesting that the photosynthetic and productivity values obtained for 2011 are representative for the environmental conditions tested and not artificially altered by the experiment. Thus, it is not likely that the additional water use in the heated plots had a substantial effect on growth and physiology during 2009.

With the exception of the eC+eT treatment relative to the control in 2011, the responses observed for A’ were similar in responses to those observed for AGB. It is interesting, however, that the response of SY to the various treatments did not follow the responses of A’ and of AGB. In 2009 the AGB was significantly higher in the eT+eC treatments relative to the control but the SY for this treatment did not differ statistically from the control. The statistically significant increase in SY for the eT+eC relative to the eT treatment was the only statistically significant difference in SY in 2009. This suggests that, in this year, the increase in [CO₂] offset the losses typically associated with an increase in temperature. In 2011, there were no differences in SY between the eT+eC and the eT treatments which implies that the benefit of both factors together are not universal. In 2011, increases occurred for both AGB and SY in the eC treatment relative to the control but the loss in SY was amplified in relation to the loss in AGB for both heated treatments (Table 3).

Within a growing season, increasing temperature appears to reduce yields (Table 3, Fig. 6), despite the 13.5% decrease in 2009 not being statistically different from the control (p<0.15). Recent multiple regression analyses of historical yield data and growing season temperatures indicate a negative relationship, meaning that yields of soybean are depressed in warmer years (Lobell & Field 2007; Kucharik & Serbin 2008) a phenomena that is consistent with our findings. A separate analysis, however, suggests that increases in yields with rising temperatures are likely to occur in cooler areas (e.g., Midwestern US) while decreases in yields with temperature are to occur in traditionally warmer areas (e.g., southern US; Hatfield et al., 2011). Our data indicates that additional heating did not confer an advantage to SY, even in a year when background temperatures were much cooler than the long-term mean (2009). A problem with historical trends is that they neglect other confounding factors that cannot be controlled for, such as an interaction with an increase in [CO₂]. When the interaction between rising [CO₂] and
temperature was considered (e.g., Fig. 6), it was clear that the yield responses were not consistent with the observed photosynthetic responses and that they varied based on growing season conditions. The results from the 2009 growing season suggest that the increased [CO2] completely negated the detriment of increased temperature on yield. The 2011 growing season showed that the addition of [CO2] with higher temperatures did nothing to mitigate the influence of higher temperature. As the conditions associated with 2011 growing season are predicted to become more common (Hayhoe et al., 2010), these results suggest that the assumptions, based on theory that rising [CO2] and increasing temperature could synergistically increase yields, needs to be reassessed.

All treatments in 2009 and the elevated temperature treatment in 2011 resulted in decreases in harvest index compared to control. The decline in HI for elevated [CO2] grown soybean are consistent with a number of previous studies on soybean (Amthor et al., 1994; Heagle et al., 1998; Ziska & Bunce, 2000; Morgan et al., 2005). Similarly, the influence of increasing temperatures drive harvest indices for soybean down, with a decrease in seed size being a major factor for this response (Boote et al., 2005). All treatments in 2009 had a smaller percentage decrease on HI compared to the control in 2009 than in 2011 (Fig. 6). This is consistent with previous reports for soybean that show an accelerated decline in HI as temperatures increase (Boote et al., 2005). While higher temperatures were not directly imposed upon the eC treatments in 2011, the CO2-induced closure of gs warmed canopies above the temperatures in the control plots, as reported in previous years (Bernacchi et al., 2007). The measured canopy temperature data used in the heating control system (Supplementary Fig. 4) indicates that season-mean increases in CO2 resulted in ca 1°C warmer canopy temperatures during midday hours. This CO2-induced warming, which was apparent in 2011 but not in 2009, could potentially contribute to the larger decline in HI for the elevated [CO2] treatment relative to the control. There were marked differences in biomass components, leaf physiology, and water potential between the 2009 and 2011 growing seasons. The factors that can influence each of these processes are complex and subjected to a significant number of environmental factors. A full understanding of the drivers behind differences between these two growing seasons would need to account for these factors and require analyses that extend beyond the measurements in this paper.
Conclusion

The results from this research show that increased temperatures reduced photosynthesis, growth and yield in soybean. These responses were linked with reductions in $g_s$, although biochemical changes to photosynthesis (e.g., Rosenthal et al., submitted) likely also influence these photosynthetic results. Moreover, the combined effects of elevated [CO$_2$] and warmer temperature did not lead to significant increases in photosynthesis compared with an increase in [CO$_2$] alone. In fact, the combined increases in [CO$_2$] and temperature led to reduced photosynthesis relative to elevated [CO$_2$] alone in one of the two years. The contrasting climatic conditions over the two years of measurements played a significant role for the different photosynthetic, growth and yield rates observed. These results suggest that in future climate change conditions the interactive effects of elevated [CO$_2$] and warmer temperatures will likely not benefit soybean physiology, growth and development as predicted from theory due to overriding environmental factors.

Materials and methods:

Site description and experimental design

This experiment was conducted on soybean [Glycine max (L.) Merr., cv. Pioneer 93B15] during the 2009 and 2011 growing seasons, as part of the Temperature by Free Air CO$_2$ Enrichment (T-FACE) experiment located within the SoyFACE research facility in Champaign, IL, USA. The entire SoyFACE research farm consists of a 32 ha (80 acres) field in Illinois (40° 2' 30.49" N, 88° 13' 58.80 W, 230 m a.s.l.). Characteristics of the site and details of agriculture practices can be found in Ainsworth et al. (2004), Rogers et al. (2004), and Bernacchi et al. (2006). The experiment consisted of a randomized complete block design with four blocks to account for field topographic and soil variation. Each block contained one control and one elevated [CO$_2$] plot; each plot had a diameter of 20 m and they were separated from each other by 100 m as described previously (details in Miglietta et al., 2001). The target concentration of 585 μmol mol$^{-1}$ in 2009 and 590 μmol mol$^{-1}$ in 2011 for the elevated [CO$_2$] plots was maintained from sunrise to sunset and applied from emergence to harvest. Ambient [CO$_2$] was ~385 μmol mol$^{-1}$ in 2009 and ~390 μmol mol$^{-1}$ in 2011. The T-FACE experimental areas consisted of a 3-m-
diameter reference and heated plots nested within each of the four 20 m diameter control and elevated [CO₂] plots.

Each heated plot contained 6 infrared heaters (Salamander Aluminum Extrusion Reflector Assembly Housing for Ceramic Infrared Heaters; Mor Electric Heating Association Inc., Comstock Park, MI, USA) each fitted with four infrared heating elements (Mor-FTE 1000W, 240V heaters; Mor Electric Heating Association Inc.). The six heaters were arranged in a 3-m-diameter hexagonal pattern with a total heating area of 7.1 m² (Supplementary Fig. 3). The heaters were maintained 1.2 m above the canopy, tilted toward the center of the plot at a 45° angle as in Kimball et al. (2008). The heater output was regulated using a custom built industrial dimmer system in which two thyristors (dimmers) were controlled using one circuit board, all of which were taken from one complete dimmer assembly (Model LCED-2484, 240V, 35A; Kalglo Electronics Co., Inc., Bethlehem, PA USA). The dimmer circuit board controlled a range of output up to 24,000W of infrared heating power. The actual 0 to 240V AC output was scaled from a 0-10 V DC input signal to the dimmer. Each heated plot was controlled using a datalogger (CR1000 Micrologger; Campbell Scientific, Inc., Logan, UT USA). The datalogger used a proportional-integrative-derivative (PID) feedback control system, similar to the ones used in Kimball (2005), to maintain a ~3.5°C increase over the ambient temperature 24h/day throughout the growing seasons (Supplementary Fig. 4). The reference and heated canopy temperatures were measured using infrared radiometers (SI-121; Apogee Instruments, Inc., Logan, UT, USA) wired into the datalogger equipped with a voltage output module (SDM-CV04; Campbell Scientific Inc.). Based on the canopy temperature difference between the heated vs. reference plots the voltage output module supplied the 0-10V signal to the dimmer to maintain the target temperature rise of heated plots above the reference plots. The full experiment consisted of four treatments: control (ambient [CO₂] & ambient temperature), elevated temperature (ambient [CO₂] & +3.5°C in temperature, eT), elevated [CO₂] (585 μmol mol⁻¹ [CO₂] & ambient temperature, eC), and elevated temperature plus elevated [CO₂] (585 μmol mol⁻¹ [CO₂] & +3.5°C in temperature, eT+eC).

**Meteorological and micrometeorological data**

Temperature, humidity and solar radiation for the research site were obtained from a meteorological station associated with the Surface Radiation Network (SURFRAD, 40.05N, 88.37W, http://www.srrb.noaa.gov/surfrad/index.html), processed as described in Vanloocke et
al. (2010). Precipitation was obtained from the Willard airport station (40.04N, 88.27W, http://cdo.ncdc.noaa.gov/qclcd/QCLCD). A record for 30-year mean temperature and precipitation for Zone 5 of the Illinois climate divisions (which correspond to Champaign) was obtained from the Midwestern Regional Climate Center (MRCC, http://mrcc.isws.illinois.edu/).

**Gas exchange measurements and midday sampling**

Diurnal measurements of instantaneous $A$ and other physiological data, such as $C_i$ and $g_s$, were collected using portable open gas-exchange systems with incorporated infrared CO$_2$ and water vapor analyzer (Li-Cor 6400; Li-Cor, Inc., Lincoln, NE, USA) coupled with an integrated chlorophyll fluorometer (LI-6400-40 leaf chamber fluorometer; Li-Cor, Inc.). The infrared CO$_2$ and water vapor analyzers were zeroed and the gas exchange systems were calibrated as described in Bernacchi et al. (2006).

Gas exchange sampling occurred every two weeks throughout both growing seasons starting with the V3 vegetative developmental stage (i.e., following the emergence of the 3$^{rd}$ trifoliate) and ending with the ~R6 developmental stage (full seed) based on the development classifications of Ritchie et al. (1993). There were a total of seven measurement days in 2009 and six in 2011 (Supplementary Table 1). The measurements were taken at 2-hour intervals between 9 am to 5 pm, on 3 plants per plot from the youngest fully expanded leaves. Four research teams each using matched instrumentation conducted measurements at each time point of the diurnal in different blocks to sample all sixteen plots within 45 min. The order of sampling for each team was randomized among blocks. At the beginning of each time point measurement, Photosynthetic Photon Flux Density (PPFD; LI-190; LI-COR, Inc.) and air temperature (HMP-45C; Campbell Scientific, Inc., mounted in aspirated temperature shield model 076B; Met One Instruments, Grants Pall, OR USA) were recorded from sensors located at SoyFACE. The block temperature of the gas exchange systems were set according to ambient air temperature and adjusted to higher temperatures (ambient + 3.5 °C) for the heated plots. The sample RH were between ~50-70%. The [CO$_2$] in the gas exchange system reference chamber was set to concentrations that corresponded to the control (400 μmol mol$^{-1}$) or elevated (600 μmol mol$^{-1}$) [CO$_2$] plots. These concentrations, while above the mean concentrations in each plot, allowed for the leaf to draw CO$_2$ down in the sample chambers to closely match plot means and accounted for the difference in the control and elevated [CO$_2$] for 2009 compared to 2011. The values of $A$, $g_s$, and $C_i$ were calculated using the integrated software in the gas exchange system according to
von Caemmerer & Farquhar (1981). The daily integrated carbon uptake ($A'$) was calculated from instantaneous $A$ as described in Leakey et al. (2004).

Values for maximum carboxylation capacity ($V_{c,max}$), maximum linear electron transport through photosystem II ($J_{max}$) and respiration in the light ($R_d$) at 25°C were obtained from $A$ vs. $C_t$ curves measured within 1-2 days of each diurnal for both growing seasons (Rosenthal et al., submitted). These parameters were used to model $A$ using the leaf photosynthesis model (Farquhar et al., 1980) to determine which processes, Rubisco or RuBP regeneration, was limiting photosynthesis at each time point for all treatments. The model was corrected for measured leaf temperature using the temperature functions provided previously (Bernacchi et al. 2001, 2003b).

Percent stimulation of $A'$ per plot for each day was calculated with the plot mean values for eT vs. Control and for eT+eC vs. eC. The percent stimulation was plotted as a function of the daily maximum temperatures for the heated plot. The values used were from 4 measurement days in 2009 (DOY 197, 210, 224, 238) and 2011 (DOY 200, 214, 228, 242), selected according to similarities in the vapor pressure deficit and solar radiation and adequate canopy cover to prevent the influence of soil in the measurements of canopy temperature (LAI > 2). The intrinsic water use efficiency (iWUE) was calculated with $A$ divided by $g_s$.

Samples to determine leaf water potential (WP) were collected during the midday time point on each measurement day with five subsamples taken per plot. Three leaf tissue discs of 1.2 cm diameter were excised per plant and sealed in psychrometer chambers (C-30; Wescor, Inc., Logan, UT USA). The chambers were equilibrated in a controlled environmental growth chamber at 25°C as described previously (Leakey et al., 2006). After thermal equilibration, WP was measured using a dew point micro-voltmeter (HR-33T; Wescor) integrated into the psychrometers. Upon measuring WP, the chambers were submerged into liquid nitrogen and the potentials were recorded on the lysed plant tissue to determine osmotic potential (OP). The turgor potential (TP) was calculated as WP–OP. A calibration standard was obtained independently each year using sucrose solutions ranging in concentration from 0 to 1.60 M.

**Above-ground biomass and yield measurements**

Above-ground biomass (AGB) was obtained at the end of each growing season after full maturity (growth stage R8) was reached (DOY 267 in 2009 and DOY 298 in 2011). Five 1.5 m rows of soybeans per plot in 2009 and two 1.0 m rows per plot in 2011 were harvested by hand.
At harvest, plants had few attached leaves, so AGB included only pods and stems. These biomass components are typically used for determining harvest index. Each component was dried to constant weight (~7 days) at 65°C and weighed. The pod tissues were then run through a thresher to isolate the seeds, and the seeds were weighted to obtain seed yield (SY). The harvest index (HI) was calculated by dividing SY by AGB.

**Statistical analysis**

Photosynthesis, water potential, and iWUE were analyzed using a complete block repeated measures mixed model analysis of variance (ANOVA) using the PROC MIXED command with the Kenward-Roger method in SAS System 9.3 (SAS Institute, Cary, NC USA). The fixed effects for the seasonal analysis were: day of the year (DOY), [CO₂], temperature, temperature by [CO₂] interaction and DOY with the interaction of the other fixed effects. Statistical tests of within day differences among the treatments were analyzed separately for each day using time of day, rather than DOY, as the repeated factor in the analysis. The above-ground biomass, yield data and HI were analyzed similarly to the previous variables but without including DOY in the analysis. The differences of least square means from *t*-tests were used to compare individual treatment means, this option is integrated into the SAS System 9.3. To lower the possibility of a Type II error, the statistical significance was evaluated at alpha ≤ 0.1.
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Table 1. Annual, 6 months (from April to September) and monthly mean temperature (°C) and precipitation (mm) for a period of 30 years and for 2009 and 2011.

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<tr>
<th>Temperature (°C)</th>
<th>Apr</th>
<th>May</th>
<th>Jun</th>
<th>Jul</th>
<th>Aug</th>
<th>Sep</th>
<th>6-month mean</th>
<th>Annual mean</th>
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<tbody>
<tr>
<td>30-year mean</td>
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<td>16.4</td>
<td>21.6</td>
<td>23.5</td>
<td>22.3</td>
<td>18.5</td>
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<tr>
<td>2009</td>
<td>9.7</td>
<td>16.4</td>
<td>22.0</td>
<td>20.6</td>
<td>20.8</td>
<td>18.1</td>
<td>16.7</td>
<td>9.9</td>
</tr>
<tr>
<td>2011</td>
<td>10.6</td>
<td>16.3</td>
<td>22.3</td>
<td>26.3</td>
<td>23.1</td>
<td>17.0</td>
<td>18.2</td>
<td>11.0</td>
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<table>
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<tr>
<th>Precipitation (mm)</th>
<th>Apr</th>
<th>May</th>
<th>Jun</th>
<th>Jul</th>
<th>Aug</th>
<th>Sep</th>
<th>6 month mean</th>
<th>Annual mean</th>
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<td>30-year mean</td>
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<td>106.9</td>
<td>98.4</td>
<td>113.2</td>
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<td>2009</td>
<td>151.9</td>
<td>135.9</td>
<td>107.2</td>
<td>105.7</td>
<td>114.1</td>
<td>27.9</td>
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<tr>
<td>2011</td>
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<td>140.7</td>
<td>101.4</td>
<td>44.5</td>
<td>52.8</td>
<td>94.5</td>
<td>609.9</td>
<td>987.3</td>
</tr>
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</table>
Table 2. Seasonal complete block repeated measures analysis of variance for the daily integral of carbon uptake ($A'$), stomatal conductance ($g_s$), intercellular [CO₂] ($C_i$), water potential (WP), osmotic potential (OP), turgor pressure (TP), intrinsic water use efficiency (iWUE), above-ground biomass (AGB), seed yield (SY), and harvest index (HI) for soybean grown in a CO₂ by temperature interaction. The main effects are [CO₂] (CO₂), temperature (Temp) and day of the year (DOY). The statistically significant differences ($p<0.1$) and non-statistical significance (ns) are shown in the table. Main effects not applied are indicated with a line (―).
<table>
<thead>
<tr>
<th>% Deviation in main effects</th>
<th>% Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>eC vs. Control</td>
</tr>
<tr>
<td>CO₂</td>
<td>Temp</td>
</tr>
<tr>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>2009</td>
<td></td>
</tr>
<tr>
<td>A'</td>
<td>14.3 *</td>
</tr>
<tr>
<td>gₘ</td>
<td>-31.8 *</td>
</tr>
<tr>
<td>Cₛ</td>
<td>41.8 *</td>
</tr>
<tr>
<td>WP</td>
<td>2.0</td>
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<tr>
<td>OP</td>
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<tr>
<td>TP</td>
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<tr>
<td>iWUE</td>
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<tr>
<td>AGB</td>
<td>19.7 *</td>
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<tr>
<td>SY</td>
<td>11.7</td>
</tr>
<tr>
<td>HI</td>
<td>-6.8 *</td>
</tr>
</tbody>
</table>

2011

| A'  | 24.6 * | -12.4 * | 21.8 * | -14.8 * | 9.1 * | 28.0 * | -10.4 * |
| gₘ | -25.6 * | -37.5 * | -25.9 * | -37.7 * | -53.4 * | -25.1 * | -37.1 * |
| Cₛ | 44.0 * | -16.6 * | 45.0 * | -15.9 * | 20.2 * | 42.9 * | -17.2 * |
| WP  | 3.9 | -16.0 * | 5.2 | -14.6 * | -11.4 | 2.9 | -17.4 * |
| OP  | 0.4 | -3.0 | -0.3 | -3.7 | -2.6 | 1.0 | -2.3 |
| TP  | -2.1 | -15.8 * | 3.8 | -10.4 | -18.2 | -8.7 | -21.1 * |
| iWUE | 63.8 * | 37.9 * | 66.1 * | 39.9 * | 126.9 * | 62.2 * | 36.6 * |
| AGB | 26.0 * | -24.0 * | 38.2 * | -14.5 | -4.4 | 11.8 | -30.9 * |
| SY  | 4.4 | -33.5 * | 20.5 | -20.4 * | -32.9 * | -15.8 | -44.3 * |
| HI  | -18.0 | -12.4 * | -13.0 | -7.2 | -28.9 * | -23.4 * | -18.3 * |

Table 3. The percentage (%) deviations from control and between treatments are shown, negative values mean a reduction while positive values mean an increase in the rate of parameters. Statistically significant differences of the pairwise comparisons are indicated (*).
Figure Legends

**Figure 1.** Meteorological conditions measured during the 2009 and 2011 growing seasons. The top panels show the daily total solar radiation (black circles) and the daily precipitation (grey bars) for the 2009 (Panel A) and 2011 (Panel B) growing seasons. The bottom panels show the vapor pressure deficit (VPD, grey line), the daily mean air temperatures (black circles) and the maximum and minimum temperatures (top and bottom of the error bars) for the 2009 (Panel C) and 2011 (Panel D) growing seasons. Days in which field sampling occurred are indicated with triangles.

**Figure 2.** Daily integral of carbon assimilation ($A'$; Panels A and B), stomata conductance ($g_s$; Panels C and D) and intercellular [CO$_2$] ($C_i$; Panels E and F) for the 2009 (Panels A, C, and E) and 2011 (Panels B, D and F) growing seasons. Each day represents the daily mean values collected using gas exchange ($g_s$ and $C_i$) and each day $A'$. Seasonal means for each variable is also presented at the right of each graph. Error bars represent one SE around the mean.

**Figure 3.** Percent deviation of integrated carbon uptake ($A'$) as a function of daily maximum canopy temperature for the 2009 (Panel A) and 2011 (Panel B) growing seasons. The percent stimulation of $A'$ was calculated with the plot means between the elevated temperature treatment (eT) compared to control (black points and black line) and between the combined effect of elevated temperature and [CO$_2$] (eT+eC) compared to elevated [CO$_2$] (eC). Data was used from measurement days in each year that occurred after canopy closure and before senescence. The lines reflect second-order polynomials fitted to each comparison.

**Figure 4.** The relationship between carbon uptake ($A$) and stomatal conductance ($g_s$) per treatment for 2009 (Panel A) and 2011 (Panel C). Each point represents the plots means (three subsamples per plot) for each hourly time point measured across all days within a season for $A$ vs. $g_s$. The intrinsic water use efficiency (iWUE), calculated as $A/g_s$, for all the measurement days during 2009 (Panel B) and 2011 (Panel D). Seasonal means for each treatment in both years are presented at the right of each graph. Bars are as in Fig. 2. Error bars represent one SE around the mean.

**Figure 5.** Water potential (WP; Panels A and B), osmotic potential (OP; Panels C and D) and turgor pressure (TP; Panels E and F) for the days where measurements were done during
2009 (Panels A, C, and E) and 2011 (Panels B, D, and F). Seasonal means of WP, OP and TP for each treatment in both years are also presented in the right in each graph. Bars are as in Fig. 2. Error bars represent one SE around the mean.

**Figure 6.** Means for the above-ground biomass (AGB; Panels A and B), seed yield (SY; Panels C and D), harvest index (HI; Panels E and F) for 2009 (Panels A, C, and E) and 2011 (Panels B, D, and F). Bars are as in Fig. 2. Error bars represent one SE around the mean.
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