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Intensifying drought eliminates the expected benefits of elevated carbon dioxide for soybean

Sharon B. Gray[1†], Orla Dermody[1], Stephanie P. Klein[1†], Anna M. Locke[1†], Justin M. McGrath[1], Rachel E. Paul[1], David M. Rosenthal[1†], Ursula M. Ruiz-Vera[1], Matthew H. Siebers[1†], Reid Strellner[1], Elizabeth A. Ainsworth[1,2], Carl J. Bernacchi[1,2], Stephen P. Long[1], Donald R. Ort[1,2] and Andrew D. B. Leakey[1]*

Stimulation of C_3 crop yield by rising concentrations of atmospheric carbon dioxide ($[CO_2]$) is widely expected to counteract crop losses that are due to greater drought this century. But these expectations come from sparse field trials that have been biased towards mesic growth conditions. This eight-year study used precipitation manipulation and year-to-year variation in weather conditions at a unique open-air field facility to show that the stimulation of soybean yield by elevated $[CO_2]$ diminished to zero as drought intensified. Contrary to the prevalent expectation in the literature, rising $[CO_2]$ did not counteract the effect of strong drought on photosynthesis and yield because elevated $[CO_2]$ interacted with drought to modify stomatal function and canopy energy balance. This new insight from field experimentation under hot and dry conditions, which will become increasingly prevalent in the coming decades, highlights the likelihood of negative impacts from interacting global change factors on a key global commodity crop in its primary region of production.

Rising $[CO_2]$ this century is predicted to stimulate the yield of C_3 crops, counteracting the negative impacts of greater drought on future food production[1–3]. The mechanisms most commonly cited to explain greater yield under elevated $[CO_2]$ are (1) direct stimulation of photosynthetic CO_2 uptake and, thereby, biomass accumulation and yield; and (2) reduced stomatal conductance (g_s) driving lower crop water use and conserving soil moisture, which ameliorates yield loss to drought stress when it occurs[4–8]. Consequently, the magnitude of relative yield stimulation by elevated $[CO_2]$ is frequently predicted to increase as drought intensifies[8–10]. Soybean (*Glycine max* Merr.) is the most important oil and protein seed crop globally[11]. Soybean has also been investigated widely as a model for understanding the response of C_3 species to global change[12]. However, as with many species, experimental testing of CO_2 response in the field has occurred over a limited number of locations and growing seasons, which limits the inference space of the previously published literature to conditions with little to no drought stress[13]. In addition, theory predicts that reduced g_s at elevated $[CO_2]$ might lower canopy water use in crops with short and dense canopies, like soybean, less than in other vegetation types because of weaker coupling to the bulk atmosphere[14,15]. Given that projected crop yields and food security for the latter part of this century are highly sensitive to the magnitude of CO_2 fertilization effects[1–3], it is important to address the uncertainty about how soybean responds to elevated $[CO_2]$ under the stronger drought stress that is predicted to characterize future growing conditions.

Experimental design

This study took a two-pronged approach to determine the interaction of drought and elevated $[CO_2]$ on the productivity of field-grown soybean. (1) Year-to-year variation in the effect of elevated $[CO_2]$ on soil water content and yield was assessed over eight growing seasons in relation to natural variation in drought stress and canopy properties, including leaf area and temperature. A commercial soybean cultivar (Pioneer 93B15) was grown at the Soybean Free-Air CO_2 Enrichment (SoyFACE) facility in a replicated experiment ($N = 4$) at ambient $[CO_2]$ (376–392 ppm) and elevated $[CO_2]$ (550–585 ppm) from 2004–2011. These ranges represent increasing ambient CO_2 over the eight-year experiment and corresponding changes in the target $[CO_2]$ for fumigation to maintain consistent treatment. The mean growing season temperatures varied (19.1–23.2 °C) and total growing season precipitation varied (274–470 mm) (Supplementary Fig. 1). An additional six soybean cultivars (Dwight, HS93-4118, IA3010, LN97-15076, Loda and Pana) were also assessed in this field facility for yield response to elevated $[CO_2]$ from 2004–2008. (2) A rainfall exclusion treatment was used to manipulate water availability in combination with CO_2 treatments over three growing seasons from 2009 to 2011 (Fig. 1a). Productivity responses of Pioneer 93B15 in the rainfall exclusion experiment were assessed in relation to soil moisture and rooting dynamics, root-to-shoot signalling and leaf photosynthetic gas exchange. SoyFACE is located in the Midwestern United States, where more than 80% of the national and more than 25% of the global soybean crop is grown[16]. This location allows experimental exposure of soybean to climate change treatments in a setting that is directly relevant to agricultural production. Over 90% of soybean production in the United States is rain fed[17]. As such, this crop is susceptible to year-to-year variation in drought stress. FACE fumigation was applied using the method of Miglietta *et al.*[18], which directly releases CO_2 into the wind stream and does not cause the atmospheric turbulence that has been detected in other experiments where air blowers are used to distribute CO_2-enriched air. Furthermore, the use of FACE technology

[1]Department of Plant Biology and Institute for Genomic Biology, University of Illinois at Urbana-Champaign, Champaign, Illinois 61801, USA. [2]United States Department of Agriculture, Agricultural Research Service, Urbana, Illinois 61801, USA. [†]Present addresses: Department of Plant Biology, University of California, Davis, California 95616, USA (S.B.G.). CSIRO Plant Industry, Urrbrae, South Australia 5064, Australia (M.H.S.). United States Department of Agriculture, Agricultural Research Service, Raleigh, North Carolina 27695, USA (A.M.L.). Department of Environmental and Plant Biology, Ohio University, Athens, Ohio 45701, USA (D.M.R.). Department of Plant Science, Penn State University, State College, Pennsylvania 16802, USA (S.P.K.).
*e-mail: leakey@illinois.edu

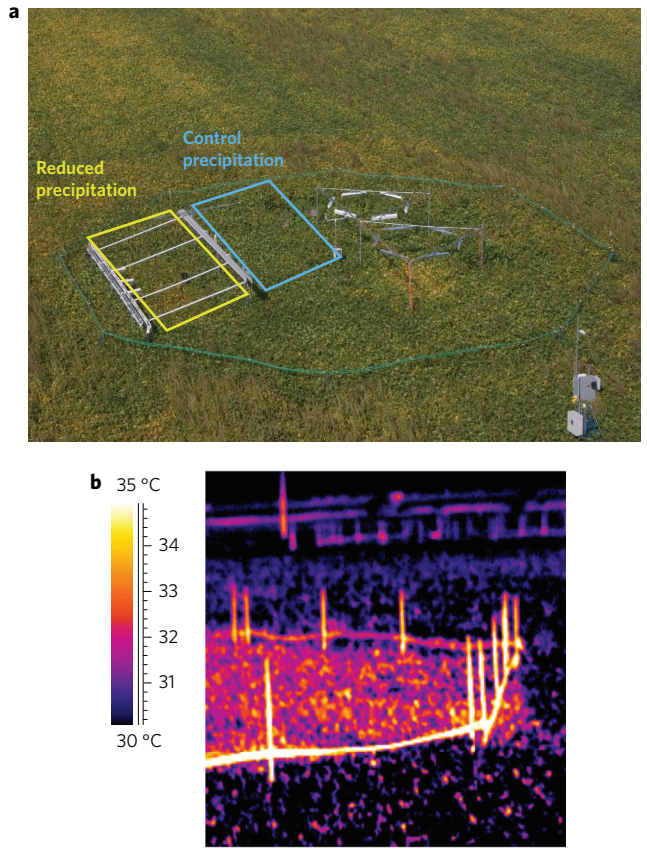

Figure 1 | SoyFACE experimental plots. a, Aerial photo of one of the eight experimental plots, in which plants were exposed to ambient or elevated [CO_2], including subplots where the crop experienced control precipitation (CP; area within blue box) or reduced precipitation (RP; area within yellow box) through rainfall interception by retractable awnings. CO_2 was released from the sections of green pipe on the upwind sides of the plot at a given moment in time. **b**, The false-colour infrared image shows stimulation of canopy temperature of soybean growing in elevated [CO_2], which is associated with reduced stomatal conductance. Panel b reproduced from ref. 62, Annual Reviews.

meant that there were no artificial restrictions on root proliferation, and soil water content throughout the rooting zone responded dynamically to experimental treatments, weather patterns and plant water use.

Elevated [CO_2] does not always conserve soil water

In contrast to the prevailing notion that elevated [CO_2] will consistently conserve soil water content, there was significant year-to-year variation in this response, depending on the duration and timing of drought events, and interactions with canopy leaf area and temperature (Figs 2 and 3). During soil drying events in four of the eight growing seasons, soil volumetric water content ($H_2O\%_{v/v}$) in elevated [CO_2] was significantly greater through most of the rooting zone (Fig. 2a,b,d,f and Supplementary Table 1). In two years, soil $H_2O\%_{v/v}$ in elevated [CO_2] was not significantly different from soil $H_2O\%_{v/v}$ in ambient [CO_2] (Fig. 2c,e; Supplementary Table 1). However, in another two years, elevated [CO_2] significantly increased surface soil $H_2O\%_{v/v}$ early in the season, but soil $H_2O\%_{v/v}$ in deeper layers became significantly lower at elevated [CO_2] in the mid- to late season (Fig. 2g,h; Supplementary Table 1). Elevated [CO_2] significantly increased the leaf area index (LAI) in every year of this study, and significantly increased daytime canopy temperature in all but two years of this study (Supplementary Tables 2 and 3). The average effect of elevated [CO_2] on soil $H_2O\%_{v/v}$ was significantly and negatively correlated with the magnitude of changes in both the maximum LAI and the daytime canopy temperature at elevated [CO_2] during the period of canopy closure (Fig. 3a,b). Although elevated [CO_2] significantly reduced midday g_s during every year of this study (Supplementary Fig. 4 and Supplementary Table 4), the average effect of elevated [CO_2] on soil $H_2O\%_{v/v}$ was not significantly correlated with the effect of elevated [CO_2] on midday g_s during the same period (Supplementary Fig. 5). It has long been predicted from theory that greater LAI and canopy temperature can counteract the reduction in canopy transpiration resulting from reduced g_s at elevated [CO_2], especially in short, dense canopies that are not always tightly coupled to the atmosphere, like soybean[14,15]. Nevertheless, the field data previously available suggested that stomatal responses are dominant and that canopy evaporation is consistently reduced at elevated [CO_2][4,5]. This study provides new experimental evidence that greater LAI and canopy temperature at elevated [CO_2] can combine to completely negate and even reverse the effects of reduced g_s on water use for this major crop in its primary area of production. Most significantly, elevated [CO_2] did not lead to consistent conservation of soil moisture throughout the soil profile during the driest and warmest years when crop stress was greatest (Figs 2 and 3). There was considerable within-season variation in the effects of elevated [CO_2] on soil $H_2O\%_{v/v}$ during these years. Elevated [CO_2] led to early-season savings in soil moisture during 2010 and 2011, but increased soil moisture depletion later in the season during the key period of reproductive development (Fig. 2g,h). This is consistent with a large stimulation of LAI by elevated [CO_2] early in the season when more water was available, translating into greater evaporative demand and drier soils later in the season. Peak LAI ranged from 5.6 to 7.1, with drier years having lower peak LAI values (Supplementary Fig. 2). Transpiration and LAI are not linearly related; increasing LAI results in increased transpiration until a threshold is reached at which self-shading and increased relative humidity in the canopy buffers the effect of additional LAI on transpiration[19]. As such, in wet years with greater baseline LAI, stimulation of leaf area by elevated [CO_2] probably did not increase water use significantly; in contrast, during dry years with lower baseline LAI, stimulation of leaf area by elevated [CO_2] had a stronger positive effect on transpiration. Consistent with this interpretation, the year-to-year variation in response of soil $H_2O\%_{v/v}$ to elevated [CO_2] was highly correlated with environmental conditions. Specifically, the effect of elevated [CO_2] on soil $H_2O\%_{v/v}$ was negatively correlated with average temperature and positively correlated with total precipitation during the period from planting until peak LAI (Fig. 3c; $r^2 = 0.86$, $p < 0.003$). This corresponds to elevated [CO_2] reducing, rather than increasing, soil $H_2O\%_{v/v}$ during warm and dry conditions, when soil water savings would have been most beneficial to the crop. This result highlights the potential for interactions among global change factors to negatively impact crop water relations in future growing conditions, which is likely to be characterized by concurrent increases in [CO_2], heatwave frequency and drought intensity[20].

Drought reduces stimulation of yield by elevated [CO_2]

Lethal drought will cause total crop failure regardless of whether plants grow at ambient [CO_2] or elevated [CO_2]. However, within the range of precipitation that supports crop production, the relative stimulation of C_3 crop yield by elevated [CO_2] has been widely assumed to increase as drought intensifies[2,8–10]. In this study, the average stimulation of yield by elevated [CO_2] across seven diverse soybean cultivars decreased as drought intensified (as the deficit of precipitation relative to potential evapotranspiration increased, Fig. 4, $p < 0.001$, $r^2 = 0.25$). Average stimulation of yield by elevated [CO_2] relative to ambient [CO_2] declined from +22% for a 'wet' growing season with a mean daily precipitation – potential evapotranspiration (P-PET) of −1.7 mm to an average stimulation of

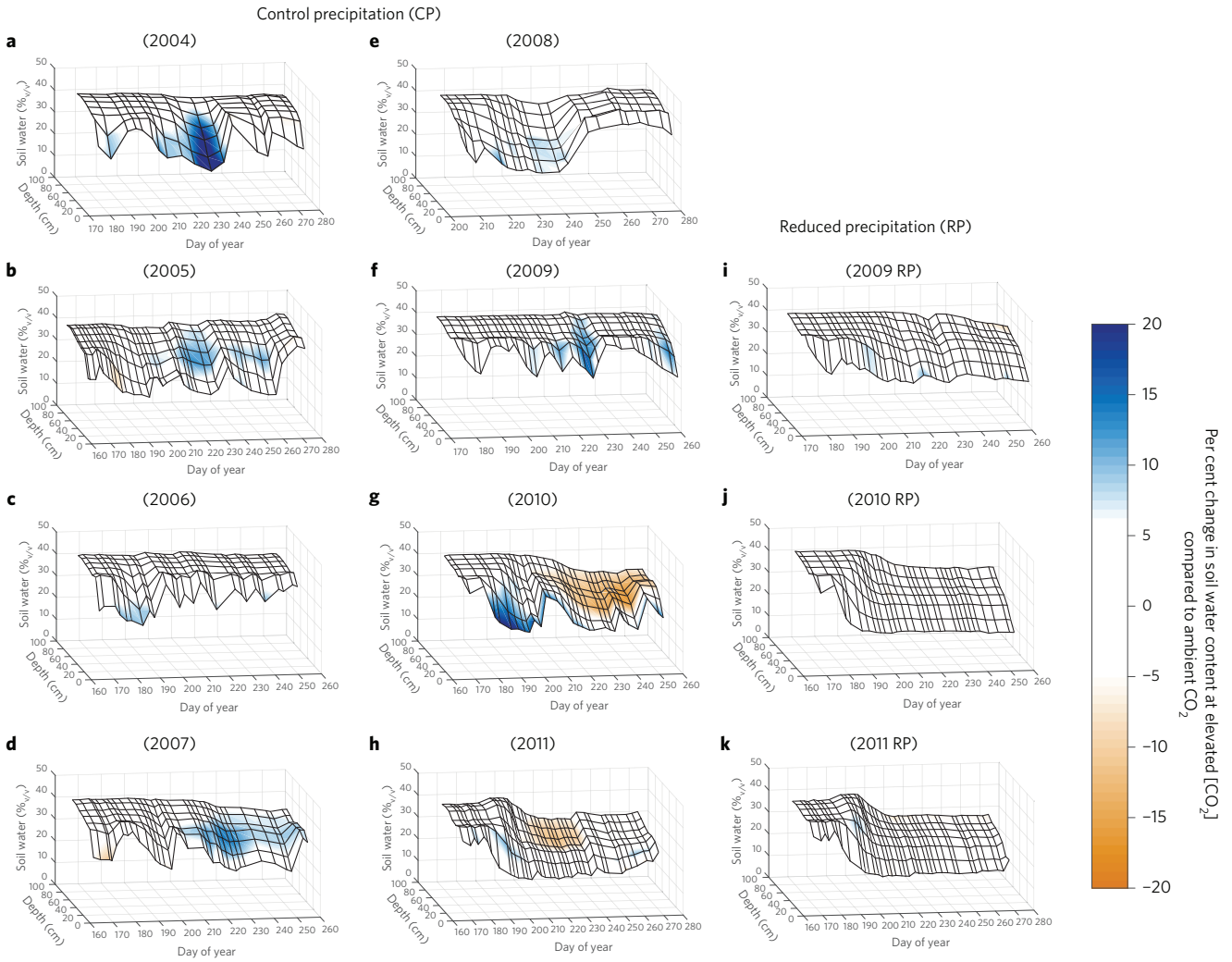

Figure 2 | Elevated CO_2 does not always conserve soil water. a–k, Vertical profile of soil volumetric moisture content ($H_2O\%_{v/v}$) in the rooting zone of soybean cv. 93B15 grown at ambient and elevated [CO_2] during the time period 2004–2011 under control precipitation (**a–h**) or reduced precipitation (**i–k**) treatments. DOY is shown on each *x* axis, soil depth (cm) is shown on each z axis and soil H_2O ($\%_{v/v}$) in ambient [CO_2] is shown on the *y* axis. The colour scale represents the effect of elevated [CO_2] on $H_2O\%_{v/v}$, with blue representing greater $H_2O\%_{v/v}$ in elevated [CO_2] and tan representing lower $H_2O\%_{v/v}$ in elevated [CO_2]. Statistical results are reported in Supplementary Tables 1 and 6.

+1% for a 'dry' growing season with a mean daily P-PET of −3.3 mm (Fig. 4). Genotypic variation in the elevated CO_2 by drought interaction effect on yield was observed, but could not be mechanistically explained with the available data. Multiple factors are likely to be involved, including variation in developmental timing among cultivars, which may have altered the synchrony between periods of peak drought stress and key reproductive periods that are sensitive to stress, for example pod set[21,22]. Nonetheless, the observation that the collective yield response to elevated [CO_2] of diverse soybean cultivars diminished with increasing drought stress in the field, and within the current climatic range of the world's primary production region for soybean, represents a major departure from the prevalent expectation.

Elevated [CO_2] does not protect plants from drought stress

The mechanisms driving soybean response to the interactive effects of elevated [CO_2] and drought were further studied in a three-year precipitation manipulation experiment, in which plants were exposed to control levels of precipitation (CP) or reduced levels of precipitation (RP) as a split-plot factor within existing ambient and elevated [CO_2] plots (Supplementary Methods; Fig. 1A). This resulted in four treatment combinations: ambient [CO_2] combined with control precipitation (AC–CP); ambient [CO_2] combined with reduced precipitation (AC–RP); elevated [CO_2] combined with control precipitation (EC–CP); and elevated [CO_2] combined with reduced precipitation (EC–RP). The reduced precipitation treatment applied in 2009–2011 was superimposed on the year-to-year variation in climate of increasingly warm and dry conditions over the three years (Supplementary Table 5). This interception of precipitation extended the range of environmental conditions experienced by the crop in the experiment to include years that were comparable with the driest years that have occurred at this location in the last 110 years (Supplementary Fig. 5).

The precipitation manipulation experiment revealed three mechanisms, which in combination drive greater yield loss of soybean to drought at elevated [CO_2]: (1) elevated [CO_2] did not lead to greater soil $H_2O\%_{v/v}$ in the RP treatment in any year (Fig. 2i–k; Supplementary Table 6); (2) nitrogen fixation may be impaired when soybean experiences elevated [CO_2] and drought simultaneously[23]; and (3) elevated [CO_2] enhanced the sensitivity of leaf photosynthetic gas exchange to soil drying, with increasing concentration of abscisic acid ([ABA]) in the xylem causing greater decreases in leaf internal [CO_2] in elevated [CO_2] compared with ambient [CO_2] (Fig. 5; Supplementary Table 7).

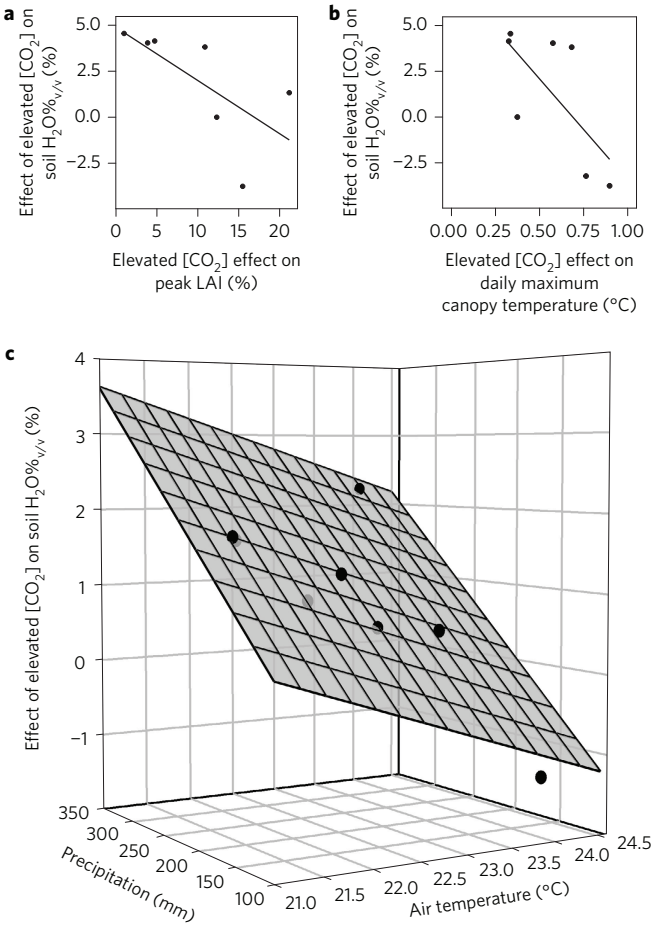

Figure 3 | Canopy traits and climate determine CO_2 effect on soil water. **a**,**b**, Linear regression of the average effect of elevated [CO_2] on soil $H_2O\%_{v/v}$ after canopy closure versus the effect of elevated [CO_2] on maximum LAI ($r^2 = 0.46$; $p = 0.095$) (**a**), and the seasonal average daily maximum effect of elevated CO_2 on canopy temperature (°C) (**b**) for soybean cv. 93B15 grown during the time period 2004–2011 ($r^2 = 0.47$, $p = 0.087$). **c**, Multiple regression of average effect of elevated [CO_2] on soil $H_2O\%_{v/v}$ versus air temperature and precipitation during the period from planting to maximum LAI (adjusted $r^2 = 0.86$; $p < 0.003$; % effect of elevated [CO_2] on soil H_2O = 7.56 + 0.01 × precipitation − 0.41 × air temperature).

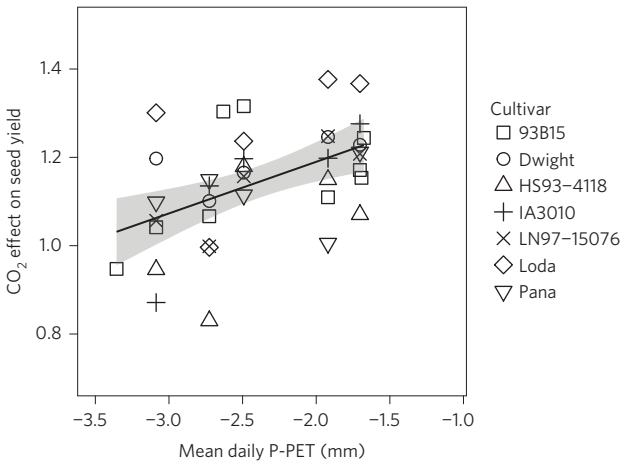

Figure 4 | Stimulation of yield by elevated [CO_2] diminishes with drought. Linear regression of the ratio of seed yield in elevated [CO_2] relative to ambient [CO_2] versus June–August precipitation minus potential evapotranspiration (P-PET; mm) for seven cultivars of soybean ($r^2 = 0.25$; $p = 0.001$; yield response = 0.12 × (P-PET) + 1.42). Data are shown for all cultivars grown at ambient [CO_2] and elevated [CO_2] under control precipitation in the 2004–2008 growing seasons, as well as 93B15 grown at ambient [CO_2] and elevated [CO_2] with control precipitation or reduced precipitation in 2010 and 2011. The line represents the line of best fit ±95% confidence intervals.

Elevated [CO_2] does not improve plant access to soil water

Consistent with earlier reports of greater root biomass or root length in elevated [CO_2][12,24], soybean root length density (RLD) was greater under elevated [CO_2] in the current study (Supplementary Fig. 6 and Supplementary Table 8). However, greater RLD occurred primarily in shallow to mid-depth soils where the soil was often dry. Combined with the lack of consistent conservation of soil moisture by elevated [CO_2] during the precipitation manipulation experiment, this RLD response meant that, despite being larger, the root system of soybean was in contact with soil of the same or slightly lower $H_2O\%_{v/v}$ in elevated [CO_2] than in ambient [CO_2] (Supplementary Fig. 7 and Supplementary Table 9). This result contrasts with the suggestion that greater allocation of carbon to roots at elevated [CO_2] might support deeper rooting and access to additional water resources that could be exploited to avoid drought stress in this crop species[25]. Furthermore, increased root length in shallow soil layers in elevated [CO_2] contributed to a shift in the distribution of root nodules to shallow, dry soils in the EC–RP treatment[23]. Specifically, 48% of the root nodules counted in the EC–RP treatment were found in dry soil (20–30% soil H_2O $\%_{v/v}$), whereas only 7–14% of nodules were found in such dry soils in other treatments. Dry soil in the immediate proximity of nodules significantly impairs nitrogen fixation activity[26]. Dry soil surrounding root nodules in the EC–RP treatment was associated with reduced leaf and seed nitrogen content, despite a substantial increase in the number of nodules produced, which suggests impairment of nitrogen fixation and an additional carbon expense[23].

Elevated [CO_2]-grown plants show stronger response to ABA

ABA is a critical signal that leads to the closure of stomata in response to drying soil[27]. Both xylem sap and leaf [ABA] increased significantly as soil $H_2O\%_{v/v}$ decreased, and they did so equally in ambient [CO_2] and elevated [CO_2] (Supplementary Fig. 8A,B and Supplementary Table 10). Although the increase in xylem sap [ABA] and leaf [ABA] that occurred in response to drying soil did not vary with CO_2 treatment, elevated [CO_2] altered the relationship between photosynthetic gas exchange and xylem [ABA]. When soils were wet and xylem [ABA] was low, stomatal conductance (g_s) was high and the leaf intercellular [CO_2] (C_i) was significantly greater in elevated [CO_2] than in ambient [CO_2] (Fig. 5a and Supplementary Fig. 9). These responses when soils were wet led to significant stimulation of net photosynthetic CO_2 uptake (A) by elevated [CO_2] (Fig. 5b and Supplementary Fig. 10), as is typically observed for soybean and other C_3 species[7,28]. As soils dried and xylem sap [ABA] increased, C_i decreased in response to stomatal closure. Unexpectedly, the reduction in g_s (Supplementary Fig. 9) and C_i (Fig. 5A and Supplementary Fig. 9) associated with drying soils was often significantly greater at elevated [CO_2] than at ambient [CO_2] (Supplementary Table 11). This differential sensitivity to soil drying was sufficiently strong to eliminate the difference in C_i between ambient [CO_2] and elevated [CO_2] when xylem sap [ABA] was greatest (Fig. 5a). Consequently, the usual stimulation of leaf-level A by elevated [CO_2] was greatly diminished or even eliminated during periods of rapid drying, such as day of year (DOY) 217 in 2011 (Fig. 5c and Supplementary Fig. 10). Further work is needed to determine if these responses were a result of greater stomatal sensitivity to [ABA] at elevated [CO_2], or some other factor such as altered

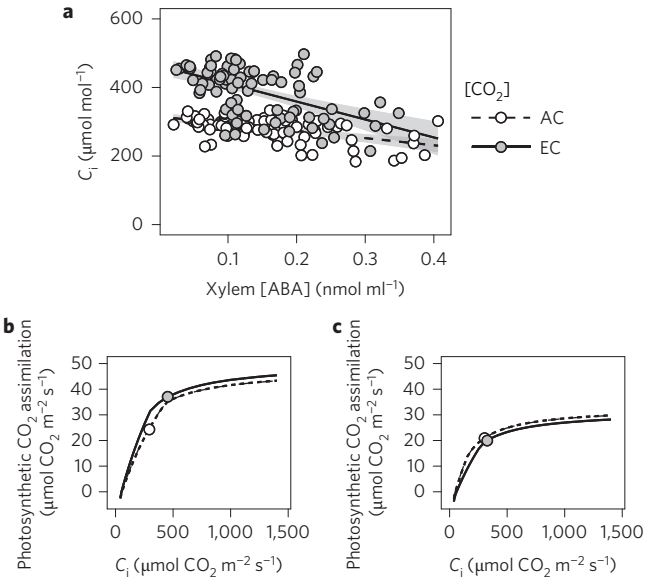

Figure 5 | Stimulation of C_i and photosynthesis by elevated [CO_2] decreases as xylem ABA increases. a, Linear regression of leaf intercellular [CO_2] (C_i, μmol mol^{-1}) versus xylem ABA concentration (nmol ml^{-1}) for soybean cv. 93B15 grown at ambient [CO_2] (open circles with dashed line; $R^2 = 0.28$) and elevated [CO_2] (grey circles with solid line; $R^2 = 0.30$) during the time period 2009–2011, including 95% confidence intervals. Statistical analyses are shown in Supplementary Table 7. **b**,**c**, Curves of net photosynthetic CO_2 assimilation (A) response to C_i, including operating C_i measured in the field for a representative ambient [CO_2] plot (open circles with dashed line) and a representative elevated [CO_2] plot (grey circles with solid line) experiencing normal water availability (**b**) and experiencing drought stress (**c**).

ABA delivery to guard cells. There are a number of chemical signals that can interact with ABA signalling and thus alter ABA-induced stomatal closure, including ethylene and cytokinin[29]. Among the chemical signals that may interact with ABA signalling, ethylene biosynthesis is responsive to elevated CO_2 in soybean leaves[30], and elevated CO_2 and nitrogen status had significant interactive effects on the xylem and leaf cytokinin content of cotton[31]. Both xylem pH and leaf water potential are known to be able to modulate the magnitude of g_s responses to xylem [ABA][27]. But, under the field conditions of the present study, elevated [CO_2] did not affect xylem pH, nor did it affect leaf water potential consistently or in the direction required to enhance stomatal sensitivity to ABA (Supplementary Fig. 8C and Supplementary Table 12). There was also no evidence that growth at elevated [CO_2] significantly altered the response of photosynthetic capacity (Supplementary Fig. 10 and Supplementary Table 13) or hydraulic conductance[32] to drought in this experiment. Altered stomatal sensitivity to ABA under elevated [CO_2] was proposed by Raschke *et al.*[33] and Bunce[34] who found that elevated [CO_2] enhanced stomatal response to ABA in *Xanthium strumarium* and soybean, respectively. In contrast, Mansfield[35] also tested *X. strumarium* but found that stomata of the same species decreased their aperture in response to ABA consistently at 0–500 ppm CO_2. These previous studies were conducted by injecting ABA into the petiole, or feeding ABA through a cut petiole, but our experiment demonstrates that across a range of naturally occurring xylem [ABA] under field conditions the leaves of elevated [CO_2]-grown soybean responded more sensitively to this signal of drought stress compared to ambient [CO_2]-grown plants. This phenomenon is likely to significantly alter whole-plant carbon balance responses to the interactive effects of elevated [CO_2] and drought as drought intensity increases in the future.

Conclusion

Two mechanisms commonly cited to justify the prediction that elevated [CO_2] will ameliorate crop drought stress in the future are (1) that elevated [CO_2] will conserve soil moisture; and (2) [CO_2] will be elevated at the site of Rubisco, as inferred from greater C_i in elevated [CO_2][8,10]. In this study, neither of these responses was sustained as drought intensified. Reduced g_s did not conserve soil moisture when it would have most benefitted soybean. Instead, interactions with weather and the indirect effects of elevated [CO_2] on plant water use via greater canopy temperature and LAI offset reductions in g_s to result in equivalent or lower soil $H_2O\%_{v/v}$ under elevated [CO_2] during the hottest and driest years. Greater stomatal closure in response to drying soils under elevated [CO_2] ultimately eliminated the stimulation of C_i required for enhanced carbon assimilation at elevated [CO_2]. In addition, negative effects of drought in combination with elevated [CO_2] were observed on nodulation patterns and tissue nitrogen content[23]. These combined limitations on carbon, water and nutrient relations provide a mechanistic basis to explain the decreasing stimulation of soybean yield by elevated [CO_2] as drought intensified (Fig. 4).

This multi-year study of the combined impacts of elevated [CO_2] and drought under field conditions in the world's most productive agricultural region demonstrates that the benefits of elevated [CO_2] for soybean are progressively eliminated as drought intensifies. As the frequency of heat waves and precipitation extremes is projected to increase during this century[20], the effect of elevated [CO_2] during the hottest and driest years included in this study may be our best available indicator of future crop performance. This highlights the potential for complex interactions among abiotic factors of global change to negatively impact our ability to meet future demand for primary foodstuffs, and the urgency of the need to develop adaptive strategies.

Methods

Field site and experimental design. This study used the SoyFACE facility in Champaign, IL (40°02′ N, 88°14′ W) (http://www.igb.illinois.edu/soyface) to grow soybean in replicated plots at either ambient atmospheric [CO_2] or elevated atmospheric [CO_2] under fully open-air conditions. The SoyFACE field site and associated management practices have been thoroughly described in previous publications[36]. Briefly, SoyFACE is located on 32 ha of farmland where soybean and maize (*Zea mays*) are each planted on 16 ha and rotated annually. The organically rich and deep soil (Drummer–Flanagan) at the site is typical of central Illinois. In common with much of the region, the field is tile drained and not irrigated. *Glycine max* (L.) Merr. cv. 93B15 (Pioneer Hi-Bred International) was grown at 0.38 m row-spacing in the 2004–2011 growing seasons. In each year, soybean was studied in four plots at ambient [CO_2] and four plots at elevated [CO_2]. The methods used to assess soil moisture, yield, biomass, LAI, leaf gas exchange, canopy temperature, leaf and xylem sap [ABA] and leaf water potential are described in subsequent sections.

FACE technology[18] was used to fumigate plants with elevated [CO_2] during daylight hours from emergence until harvest. The target concentration for elevated [CO_2] treatment was 550 ppm in 2004–2008 and increased to ~585 ppm in 2009–2011 in order to maintain treatment effects as ambient [CO_2] increased over the same period from 377 to 392 ppm.

In 2009–2011, in addition to CO_2 treatments, which were applied at the whole-plot level, soybean cv. 93B15 was exposed to either CP or RP in a split-plot design. This resulted in the four treatment combinations: AC–CP, AC–RP, EC–CP and EC–RP. In RP plots, rainfall was intercepted using modified Solair motorized retractable fabric awnings (Glen Raven Inc.), which where mounted on lightweight metal scaffolding[23]. RP and CP subplots were 8 m long × 4 m wide. A rubber subsoil barrier was installed to a depth of 1 m surrounding the RP treatment plot to prevent lateral flow of soil water from neighbouring plots. Sampling was conducted at least 0.5 m from the edge of the plot to avoid edge effects, and to avoid shade cast by the metal scaffolding. A weather station located at the field site relayed a signal to the awnings to deploy when the following conditions were met: precipitation was detected, wind speed was less than 10 m s^{-1} and light levels were below 50 μmol m^{-2} s^{-1}. These conditions for awning deployment occurred predominantly at night, and allowed interception of significant amounts of rainfall without disrupting daytime growth or fumigation conditions. Precipitation that was intercepted by awnings was routed through corrugated drainpipes and released at least 20 m away from the treatment plots. Data on awning performance, including growing season precipitation in CP and RP treatments, rainfall interception

efficiency and estimates of sunlight intercepted by awnings are included in Supplementary Table 5.

To assess the interactive effects of elevated [CO_2] and drought on the yield of a broader range of germplasm, the cultivars Dwight, HS93-4118, IA3010, LN97-15076, Loda and Pana were also grown at 0.38 m row spacing from 2004–2008 at ambient [CO_2] and elevated [CO_2] ($N = 4$). Soybean cv. 93B15 was planted across at least half of each total plot area in all years, and Dwight, HS93-4118, IA3010, LN97-15076, Loda and Pana each occupied 2.3–16.2 m^2 (ref. 37).

Climate data. Air temperature and photosynthetic photon flux density (PPFD) were obtained from a weather station located at the SoyFACE field site. Precipitation data for the 2004–2008 growing seasons were obtained from a weather station located at Willard Airport in Champaign, IL, approximately 1.5 km from the SoyFACE field site[38]. Precipitation data for 2009–2011 were collected at the SoyFACE field site using tipping bucket rain gauges (RainWise Inc.), which were located in each plot, and for these three years, growing season precipitation measured by these gauges was within 10 mm of the value recorded at the nearby Willard Airport meteorological station. Daily average potential evapotranspiration data were obtained from the Illinois Climate Network (http://www.isws.illinois.edu/warm/datatype.asp), located approximately 5 km from the SoyFACE field site. Potential evapotranspiration data were merged with the precipitation data described above to calculate daily values of P-PET. Seven day running average values were then calculated for P- PET and plotted against DOY, along with daily precipitation and air temperature values (Supplementary Fig. 1). To put the weather conditions of the 2004–2011 growing seasons into historical context, the average temperature and total precipitation for June–September were compared with the historical record going back to 1901 for Urbana, IL, obtained through the Illinois State Water Survey (http://www.isws.illinois.edu/data/climatedb/choose.asp?stn=118740; Supplementary Fig. 5).

Soil $H_2O\%_{v/v}$. The vertical distribution of soil $H_2O\%_{v/v}$ in the experimental plots of soybean cv. 93B15 was determined as previously described[23] using a capacitance probe (Diviner-2000). Measurements were made every 2–8 days, with the exception of one 12 day gap in 2004. Within each plot (ambient or elevated [CO_2] from 2004–2008; AC–CP, AC–RP, EC–CP or EC–RP in 2009–2011) measurements were made at two locations within crop rows and two locations between crop rows. At each location, measurements were made at 10 cm depth increments ranging from 5 to 105 cm soil depth. Raw data obtained from the capacitance probe were calibrated against gravimetric data for the SoyFACE site as described by previously[39]. Minirhizotron analysis at SoyFACE in 2009–2011 demonstrated that 89% of the total root length was located at 5–75 cm soil depth (Supplementary Fig. 6), so analysis of soil $H_2O\%_{v/v}$ data was restricted to these depths. Soil moisture data for 2010 were previously reported[23].

Root length and distribution. Following planting and before seedling emergence in 2009–2011, four minirhizotron tubes of cellulose acetate butyrate with a 2 inch (5.08 cm) internal diameter (Bartz Technology Corp.) were installed at 30° from vertical to a soil depth of 90 cm in each treatment plot of soybean cv. 93B15 using a tractor-mounted Giddings probe (Giddings Machine Co.)[23]. Minirhizotron tubes were placed adjacent to soil moisture access tubes, and with the same distribution and placement relative to crop rows: two tubes in each plot were located within soybean rows, and two tubes in each plot were located between soybean rows. To exclude light and water, minirhizotron tubes were wrapped heavily with tape above the soil surface and the ends were covered using aluminium cans. Images were collected along the upper surface of each tube every 2 weeks using a BTC-100× minirhizotron video microscope and a BTC I-CAP Image Capture System (Bartz Technology Corp.). Images were collected at 1.3 cm depth intervals for a total of ~80 images per tube per measurement date. For each image, root length and diameter were manually traced using the WinRHIZO Tron MF manual root measurement program (Regent Instruments) and data were combined with soil viewing area estimates and a standard estimate of depth of view (2 mm)[40] to estimate RLD (cm root cm^{-3} soil). Collectively, over 81,000 minirhizotron images were analysed from the 2009–2011 growing seasons.

Yield. In 2004–2008, the seed yield response to elevated [CO_2] of soybean cultivars 93B15, Dwight, HS93-4118, IA3010, LN97-15076, Loda and Pana was assessed as described previously[37]. In 2009–2011, seed yield response of 93B15 to the interaction of elevated [CO_2] and drought treatments was determined from undisturbed plots that were pre-marked. Harvest plots were 0.32 m^2 in 2009 and 1.524 m^2 in 2010 and 2011. Plants were cut close to the ground surface, divided into stems and pods and stored in a forced-air oven at 65 °C for a minimum of 5 days before being weighed both before and after threshing to separate seeds from pod casings. In 2009, the 93B15 samples were compromised after threshing, so final seed yield data could not be reported in Fig. 4. Additionally, in CP plots in 2010, unusually high precipitation in June resulted in high June–Aug P-PET, and this data point was determined to be an outlier with a Cook's distance value greater than 1, and so was not included in the regression analysis of seed yield and P-PET. Of the eight growing seasons of yield data analysed here, results were previously published from four growing seasons for Pioneer 93B15 (ref. 41) and five growing seasons for other cultivars[37].

Leaf area index. The LAI was measured as previously described[42]. Briefly, the LAI was measured using a plant canopy analyser (LAI-2000, Li-Cor Biosciences) in diffuse light conditions on cloudy days or within 1 h of sunrise or sunset. Within each plot, the LAI was measured in two to six locations. Measurements were made every 6–17 days, with the exception of a 36 day gap in 2006, for a total of 5–16 measurement dates each year. Multiple instruments were used on each measurement day to allow for data to be collected rapidly under consistent light conditions. Tests were regularly performed to compare variation in results among instruments used in a common plot. LAI data collected during 2011 were excluded from further analysis because the analysers used in that year failed this test. LAI for 2004[43], 2005[44], 2006[41] and 2007[41] were previously reported.

Leaf gas exchange. *In situ* leaf-level gas exchange measurements were made using portable gas exchange systems (LI-6400; LI-COR), as previously described[36]. Measurements were made in high light conditions, typically at midday, between 11:30 and 16:30 on four to six dates each year. During the 2009–2011 growing seasons, measurements were made every 2 h, 9:00 to 17:00. The light, temperature and relative humidity conditions at the top of the canopy were reproduced in the gas exchange cuvette. In each plot, three plants were randomly selected and one mature leaflet at the top of the canopy from each of these plants was measured. The equations of von Caemmerer and Farquhar[45] were used to calculate photosynthetic carbon assimilation rate (A), stomatal conductance (g_s) and leaf internal [CO_2] (C_i). Midday g_s values were used in analysis of the eight-year record from control precipitation conditions (Supplementary Fig. 4), midday C_i values were used in regression analysis with ABA data (Fig. 5) and daily average gas exchange values were used in ANOVA testing the effects of [CO_2] and precipitation treatments on gas exchange parameters in 2009–2011 (Supplementary Fig. 9 and Supplementary Table 11). Leaf photosynthetic gas exchange data for 2004[28], 2005[46], 2006[46] and 2007[47] were previously reported.

***In vivo* estimation of biochemical limitations to photosynthesis.** *In vivo* values of maximum carboxylation capacity ($V_{c,max}$) and maximum linear electron transport through photosystem II (J_{max}) were estimated from photosynthesis (A) vs. sub-stomatal [CO_2] (C_i) measurements as described previously[48] using an open gas exchange system (LI-6400, LI-COR) on six days in 2009 and five days in 2010 and 2011. Before dawn, the petioles of the uppermost fully expanded leaves were cut and immediately submerged in water. Leaves were returned to the laboratory within 30 min, petioles were re-cut and kept under water and low light (ca. <50 μmol m^{-2} s^{-1}). Photosynthesis and stomatal conductance of detached soybean leaves collected in this manner are similar to those measured in the field[48–50]. Fifteen minutes before measurements, leaves were pretreated with high light (ca. 1,000 μmol m^{-2} s^{-1}). Leaves were then placed in the measurement cuvette and allowed to reach steady-state photosynthesis at their growth [CO_2] (385 ppm or 585 ppm [CO_2]) at a saturating light level of 1500 μmol m^{-2} s^{-1}. The petioles of all leaves remained submerged in water throughout the measurements. Once steady-state A was reached, photosynthesis was recorded at the growth [CO_2], then [CO_2] was decreased stepwise to 50 μmol mol^{-1}, increased again to the growth [CO_2] and then increased stepwise to 1500 μmol mol^{-1}. A minimum of 11 data points were collected for each leaf. The A vs. C_i data were fitted to the biochemical model of photosynthesis and solved for $V_{c,max}$, J_{max} and R_d (where R_d represents mitochondrial respiration rate in the light (μmol m^2 s^{-1})), following the methods outlined by previously[51]. A vs. C_i data for control precipitation treatment in the 2009–2011 growing seasons were previously published[48].

Canopy temperature measurement. As described previously[5], the canopy surface temperature was measured using infrared thermometers (IRT-P in 2004–2006 and SI-111 in 2007–2011) that were calibrated before each growing season[52]. They were placed 1 m above the canopy in each plot. To limit the contribution of soil surface temperature to the canopy temperature reading, canopy temperature data were restricted to the period of time when LAI > 3, indicating that the canopy was 'closed'. Canopy temperature measurements were made in 10 s intervals and averaged over 10 min, and relayed to and stored on a central computer. Ten-minute average data were merged with photosynthetically active radiation (PAR) sensor data to restrict analysis to daylight hours (PPFD >50 μmol m^{-2} s^{-1}). The daily average canopy temperature during daylight hours was calculated for each plot and used in statistical analysis (Supplementary Table 3). The canopy temperature data were averaged to produce hourly treatment means, which were plotted against time to demonstrate interannual and time-of-day variability in the effect of elevated CO_2 on canopy temperature (canopy temperature in elevated [CO_2] treatment – canopy temperature in ambient [CO_2] treatment; Δcanopy temperature; Supplementary Fig. 3). The seasonal average hourly Δcanopy temperature data were used to derive the daily maximum Δcanopy temperature data (Fig. 3b). Owing to problems with sensor calibrations, the 2008 canopy temperature data were unreliable and excluded from this analysis. The canopy temperatures for the 2004 and 2005 growing seasons were previously reported[5].

Leaf tissue and xylem sap sample collection. Immediately following the 13:00 gas exchange measurements during the 2009–2011 growing seasons, leaf tissue was collected from three plants in each plot for measurement of leaf ABA content and leaf starch content. In most cases, leaf tissue was collected from the same leaves that

were used for gas exchange measurements. In cases where there was not sufficient leaf area for tissue sampling, samples were also collected from the youngest, most fully expanded leaves of neighbouring plants. Leaf discs were sealed in aluminium foil, flash-frozen in liquid nitrogen, and transferred to a −80 °C freezer until analysis. Xylem sap samples were collected in parallel with the 13:00 gas exchange measurements on each date described above. In each plot, three plants were randomly chosen, and a mature leaflet at the top of the canopy was cut at the petiole and placed in a Scholander pressure chamber (Plant Moisture Systems). The chamber was pressurized until sap began to appear. To avoid contamination from cut cells at the excision surface, initial exudate was blotted off. Approximately 30 µl of xylem sap was collected with a pipette, placed in a microcentrifuge tube, flash-frozen in liquid nitrogen and stored at −80 °C until analysis.

Determination of leaf and xylem ABA content. Xylem pH was measured using a pH microelectrode (PHR-146 pH probe, Lazar Research Labs). Leaf and xylem ABA content was measured using a modification of the radioimmunoassay described previously.[53] This assay utilized a monoclonal antibody, which is specific for (+)-ABA (AFRC MAC 252, Babraham Institute). 25 µl of each xylem sap sample was added to 100 µL of MAC 252 antibody and 100 µl of ^{3}H-ABA (DL-*cis*, *trans*-[G-^{3}H] abscisic acid, GE Healthcare). As ^{3}H-ABA and endogenous xylem ABA competed to bind with the MAC 252 antibody, the amount of ^{3}H-ABA bound by the MAC 252 antibody was inversely proportional to the amount of endogenous ABA in each sample. A scintillation counter (Packard Tri-Carb 1900 Liquid Scintillation Analyzer) was used to determine the amount of ^{3}H-ABA that was bound by the MAC 252 antibody in each sample. Endogenous xylem ABA concentrations were then calculated by comparing radioactivity values from the scintillation counter with a standard curve generated with known amounts of non-labelled ABA[54]. As described previously[55,56], to ensure minimal contamination with phloem exudate, soluble carbohydrate content was measured in a subset of xylem exudate samples ($n = 40$) using a continuous enzymatic substrate assay[57]. The sucrose concentration of xylem exudate was found to be negligible (1.01 mM ± 1.05; data not shown). This was consistent with the low level of sucrose previously reported for maize xylem sap (<1–10 mM)[58], and was much lower than published sucrose concentrations for phloem exudate, which range from 207 to 900 mM, depending on the species[58–60]. This suggests that our xylem sap collection method did not result in significant contamination from phloem exudate. Leaf ABA content was measured as described for xylem sap, but rather than adding xylem sap to the MAC 252 antibody, an extract was prepared using lyophilized leaf tissue in ddH_2O, and 50 µl of the sample extract was added to the MAC 252 antibody.

Water potential. To measure the leaf water potential, samples were collected from three plants from each plot on four or five dates per growing season. Samples were collected at midday from the field, corresponding to midday gas exchange measurements. Five leaf discs of approximately 1.2 cm in diameter were collected from each plant and immediately sealed in psychrometer chambers (C-30; Wescor, Inc.). As previously described[61], samples were equilibrated at 25 °C, and an integrated dew-point micro-voltometer (HR-33T; Wescor) was used to measure water potential in each psychrometer chamber. Following water potential measurements, tissue was lysed by submerging psychrometer chambers in liquid nitrogen, and micro-voltometers were again used to measure osmotic potential. A sucrose standard curve was used to calculate water potential and osmotic potential from raw values and turgor pressure was calculated as water potential – osmotic potential.

Statistical analysis. All statistical analyses were performed on plot means or subplot means ($n = 4$). For soil volumetric water content data for 2004–2011 in control precipitation plots, each year was analysed separately, and within years each 10 cm depth category was analysed in a separate mixed-model, repeated measures ANCOVA. Saturated soil water content at each depth at the beginning of the growing season was treated as a covariate, CO_2 treatment and date were treated as fixed effects, and block as well as the block × date interaction were treated as random effects. To analyse soil volumetric water content data from the precipitation manipulation experiment, analysis was conducted as described above, but direct effects of precipitation treatment as well as interactive effects of precipitation treatment with [CO_2] and date were also considered fixed effects. LAI and canopy temperature data, as well as g_s data for control precipitation treatment for each year were analysed in separate mixed-model ANOVAs where CO_2 treatment and measurement date were considered fixed effects and block was considered a random effect. For g_s and canopy temperature data, repeated measures ANOVAs were used. ANOVA and ANCOVA analyses were conducted using the mixed procedure of SAS (SAS ver. 9.3).

Multiple regression analysis used the average daily temperature and the sum of precipitation during the period of canopy development, correlated with the average % effect of elevated [CO_2] on soil $H_2O\%_{v/v}$ during the same time (planting – peak LAI; Fig. 3c). The average % effect of elevated CO_2 on soil $H_2O\%_{v/v}$ during the period when the canopy was closed (LAI >3) was correlated with the % effect of elevated [CO_2] on maximum LAI, the average daily maximum effect of elevated [CO_2] on canopy temperature when LAI was greater than 3 using simple regressions (Fig. 3a,b). Average % effect of elevated [CO_2] on soil $H_2O\%_{v/v}$ during the period when the canopy was closed (LAI >3) was also regressed against the % effect of elevated [CO_2] on midday stomatal conductance (g_s) during the period of canopy closure (Supplementary Fig. 5). Regression analyses were conducted using the SAS regression procedure (SAS ver. 9.3). To avoid type II errors, treatment effects were considered statistically significant at $P < 0.1$, as in previous studies[23,28,61].

Photosynthetic gas exchange data from the precipitation manipulation experiment were analysed using mixed-model ANOVA (proc mixed; SAS ver. 9.3). For each parameter, daily mean values for each plot were used in the ANOVA. [CO_2], precipitation treatment and sampling date as well as their interactions were considered fixed effects, and block was considered a random effect. Xylem ABA and xylem pH data were analysed using repeated measures mixed-model analysis of variance, where the measurement date was treated as a repeated measure, direct and interactive effects of [CO_2], precipitation treatment and date were considered fixed effects, and block and block × CO_2 were treated as random effects (proc mixed; SAS ver. 9.3). Regression analysis was used to analyse the relationship of leaf ABA (Supplementary Fig. 8A), xylem ABA (Supplementary Fig. 8B) and xylem pH (Supplementary Fig. 8C) with soil $H_2O\%_{v/v}$, CO_2 treatment and CO_2 × soil $H_2O\%_{v/v}$ interaction. Regression analysis was also used to analyse the effects of variation in xylem ABA on leaf intercellular CO_2 concentration (C_i; µmol mol^{-1}), as well as the direct effects of CO_2 treatment on C_i, and the interactive effects of CO_2 treatment with xylem ABA on C_i. All regression analyses were conducted on subplot mean values of ABA, C_i and soil $H_2O\%_{v/v}$ using the regression procedure of SAS (SAS ver. 9.3).

Plot mean data and statistical code for all analyses in the paper are deposited at Data Dryad (http://dx.doi.org/10.5061/dryad.g0v62).

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References

1. Parry, M. L., Rosenzweig, C., Iglesias, A., Livermore, M. & Fischer, G. Effects of climate change on global food production under SRES emissions and socio-economic scenarios. *Global Environ. Change* **14,** 53–67 (2004).
2. Porter, J. R. *et al.* in *Climate Change 2014: Impacts, Adaptation, and Vulnerability* (eds Field, C.B. *et al.*) 485–533 (IPCC, Cambridge Univ. Press, 2014).
3. Cline, W. R. *Global Warming and Agriculture: Impact Assessments by Country* Vol. 186 (Center for Global Development, 2007).
4. Kimball, B. A. & Bernacchi, C. in *Managed Ecosystems and CO_2: Case Studies Processes, and Perspectives* Vol. 187 (eds Nösberger, J., Long, S. P. *et al.*) 311–324 (Springer, 2006).
5. Bernacchi, C., Kimball, B., Quarles, D., Long, S. & Ort, D. Decreases in stomatal conductance of soybean under open-air elevation of [CO_2] are closely coupled with decreases in ecosystem evapotranspiration. *Plant Physiol.* **143,** 134–144 (2007).
6. Kimball, B. A., Kobayashi, K. & Bindi, M. Responses of agricultural crops to free-air CO_2 enrichment. *Adv. Agron.* **77,** 293–368 (2002).
7. Ainsworth, E. A. & Long, S. P. What have we learned from 15 years of free-air CO_2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO_2. *New Phytol.* **165,** 351–371 (2005).
8. Leakey, A. D. B. *et al.* Elevated CO_2 effects on plant carbon, nitrogen, and water relations: six important lessons from FACE. *J. Exp. Bot.* **60,** 2859–2876 (2009).
9. Ewert, F. *et al.* Effects of elevated CO_2 and drought on wheat: testing crop simulation models for different experimental and climatic conditions. *Agric. Ecosyst. Environ.* **93,** 249–266 (2002).
10. McGrath, J. M. & Lobell, D. B. An independent method of deriving the carbon dioxide fertilization effect in dry conditions using historical yield data from wet and dry years. *Glob. Chang. Biol.* **17,** 2689–2696 (2011).
11. Hartman, G. L., West, E. D. & Herman, T. K. Crops that feed the world 2. Soybean-worldwide production, use, and constraints caused by pathogens and pests. *Food Secur.* **3,** 5–17 (2011).
12. Ainsworth, E. A. *et al.* A meta-analysis of elevated CO_2 effects on soybean (*Glycine max*) physiology, growth and yield. *Glob. Chang. Biol.* **8,** 695–709 (2002).
13. Leakey, A. D. B., Bishop, K. A. & Ainsworth, E. A. A multi-biome gap in understanding of crop and ecosystem responses to elevated CO_2. *Curr. Opin. Plant Biol.* **15,** 228–236 (2012).
14. McNaughton, K. G. & Jarvis, P. J. Effects of spatial scale on stomatal control of transpiration. *Agric. Forest Meterol.* **54,** 279–301 (1991).
15. Field, C. B., Jackson, R. B. & Mooney, H. A. Stomatal responses to increased CO_2: implications from the plant to the global scale. *Plant Cell Environ.* **18,** 1214–1225 (1995).
16. Ash, M. Soybean and oil crops: background. *United States Department of Agriculture* http://www.ers.usda.gov/topics/crops/soybeans-oil-crops/background.aspx (2012).
17. USDA. 2012 Census Ag Atlas Maps. *National Agricultural Statistics Service* http://www.agcensus.usda.gov/Publications/2012/Online_Resources/Ag_Atlas_Maps/Crops_and_Plants/ (2012).
18. Miglietta, F. *et al.* Free-air CO_2 enrichment (FACE) of a poplar plantation: the POPFACE fumigation system. *New Phytol.* **150,** 465–476 (2001).

19. Woodward, F. I. *Global Warming and Biological Diversity* (eds Peters, R. L. & Lovejoy, T. E.) 105–123 (Yale Univ. Press, 1992).
20. Kirtman, B. *et al.* in *Climate Change 2013: The Physical Science Basis*. (eds Stocker, T. F. *et al.*) 485–533 (IPCC, Cambridge Univ. Press, 2013).
21. Liu, F., Andersen, M. N., Jensen, C. R. Loss of pod set caused by drought stress is associated with water status and ABA content of reproductive structures in soybean. *Funct. Plant Biol.* **30,** 271–280 (2003).
22. Tardieu, F. Any trait or trait-related allele can confer drought tolerance: just design the right drought scenario. *J. Exp. Bot.* **63,** 25–31 (2012).
23. Gray, S. B. *et al.* Minirhizotron imaging reveals that nodulation of field-grown soybean is enhanced by free-air CO_2 enrichment only when combined with drought stress. *Funct. Plant Biol.* **40,** 137–147 (2013).
24. Pritchard, S. G. & Rogers, H. H. Spatial and temporal deployment of crop roots in CO_2-enriched environments. *New Phytol.* **147,** 55–71 (2000).
25. Wullschleger, S. D., Tschaplinski, T. J. & Norby, R. J. Plant water relations at elevated CO_2—implications for water-limited environments. *Plant Cell Environ.* **25,** 319–331 (2002).
26. Durand, J. L., Sheehy, J. E. & Minchin, F. R. Nitrogenase activity, photosynthesis and nodule water potential in soybean plants experiencing water deprivation. *J. Exp. Bot.* **38,** 311–321 (1987).
27. Wilkinson, S. & Davies, W. J. Drought, ozone, ABA and ethylene: new insights from cell to plant to community. *Plant Cell Environ.* **33,** 510–525 (2010).
28. Bernacchi, C. J. *et al.* Hourly and seasonal variation in photosynthesis and stomatal conductance of soybean grown at future CO_2 and ozone concentrations for 3 years under fully open-air field conditions. *Plant Cell Environ.* **29,** 2077–2090 (2006).
29. Wilkinson, S. *et al.* Plant hormone interactions innovative targets for crop breeding and management. *J. Exp. Bot.* **63,** 3499–3509 (2012).
30. Casteel, C. L. *et al.* Effects of elevated CO_2 and soil water content on phytohormone transcript induction in *Glycine max* after *Popillia japonica* feeding. *Arthropod Plant Interact.* **6,** 439–447 (2012).
31. Wong, J. W. H. *et al.* Effects of elevated [CO_2] and nitrogen nutrition on cytokinins in the xylem sap and leaves of cotton. *Plant Physiol.* **124,** 767–779 (2000).
32. Locke, A. M. & Ort, D. R. Leaf hydraulic conductance declines in coordination with photosynthesis, transpiration and leaf water status as soybean leaves age regardless of soil moisture. *J. Exp. Bot.* **65,** 6617–6627 (2014).
33. Raschke, K. Simultaneous requirement of carbon dioxide and abscisic acid for stomatal closing in *Xanthium strumarium* L. *Planta* **125,** 243–259 (1975).
34. Bunce, J. A. Effects of humidity on short-term responses of stomatal conductance to an increase in carbon dioxide concentration. *Plant Cell Environ.* **21,** 115–120 (1998).
35. Mansfield, T. A. Delay in response of stomata to abscisic acid in CO_2-free air. *J. Exp. Bot.* **27,** 559–564 (1976).
36. Rogers, A. *et al.* Leaf photosynthesis and carbohydrate dynamics of soybeans grown throughout their life-cycle under free-air carbon dioxide enrichment. *Plant Cell Environ.* **27,** 449–458 (2004).
37. Bishop, K. A., Betzelberger, A. M., Long, S. P. & Ainsworth, E. A. Is there potential to adapt soybean *(Glycine max* Merr.) to future [CO_2]? An analysis of the yield response of 18 genotypes in free-air CO_2 enrichment. *Plant Cell Environ.* **38,** 1765–1774 (2015).
38. Vanloocke, A., Bernacchi, C. & Twine, T. The impacts of Miscanthus x giganteus production on the Midwest US hydrologic cycle. *Glob. Change Biol. Bioenergy* **2,** 180–191 (2010).
39. Paltineanu, I. & Starr, J. Real-time soil water dynamics using multisensor capacitance probes: laboratory calibration. *Soil Sci. Soc. Am. J.* **61,** 1576–1585 (1997).
40. Iversen, C., Ledford, J. & Norby, R. CO_2 enrichment increases carbon and nitrogen input from fine roots in a deciduous forest. *New Phytol.* **179,** 837–847 (2008).
41. Twine, T. E. *et al.* Impacts of elevated CO_2 concentration on the productivity and surface energy budget of the soybean and maize agroecosystem in the Midwest USA. *Glob. Change Biol.* **19,** 2838–2852 (2013).
42. Dermody, O., Long, S. & DeLucia, E. How does elevated CO_2 or ozone affect the leaf-area index of soybean when applied independently? *New Phytol.* **169,** 145–155 (2006).
43. Dermody, O., Long, S. P., McConnaughay, K. & DeLucia, E. H. How do elevated CO_2 and O_3 affect the interception and utilization of radiation by a soybean canopy? *Glob. Change Biol.* **14,** 556–564 (2008).
44. Gray, S. B., Dermody, O. & DeLucia, E. H. Spectral reflectance from a soybean canopy exposed to elevated CO_2 and O_3. *J. Exp. Bot.* **61,** 4413–4422 (2010).
45. Von Caemmerer, S. & Farquhar, G. Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. *Planta* **153,** 376–387 (1981).
46. Leakey, A. D. B. *et al.* Genomic basis for stimulated respiration by plants growing under elevated carbon dioxide. *Proc. Natl Acad. Sci. USA* **106,** 3597–3602 (2009).
47. Gillespie, K. M. *et al.* Greater antioxidant and respiratory metabolism in field-grown soybean exposed to elevated O_3 under both ambient and elevated CO_2. *Plant Cell Environ.* **35,** 169–184 (2012).
48. Rosenthal, D. M. *et al.* Biochemical acclimation, stomatal limitation and precipitation patterns underlie decreases in photosynthetic stimulation of soybean (*Glycine max*) at elevated [CO_2] and temperatures under fully open air field conditions. *Plant Sci.* **226,** 136–146 (2014).
49. Bernacchi, C. J., Morgan, P. B., Ort, D. R. & Long, S. P. The growth of soybean under free air CO_2 enrichment (FACE) stimulates photosynthesis while decreasing in vivo Rubisco capacity. *Planta* **220,** 434–446 (2005).
50. Morgan, P. B., Bernacchi, C. J., Ort, D. R. & Long, S. P. An in vivo analysis of the effect of season-long open-air elevation of ozone to anticipated 2050 levels on photosynthesis in soybean. *Plant Physiol.* **135,** 2348–2357 (2004).
51. Long, S. P. & Bernacchi, C. J. Gas exchange measurements, what can they tell us about the underlying limitations to photosynthesis? Procedures and sources of error. *J. Exp. Bot.* **54,** 2393–2401 (2003).
52. Triggs, J. *et al.* Free-air CO_2 enrichment effects on the energy balance and evapotranspiration of sorghum. *Agricult. Forest Meteorol.* **124,** 63–79 (2004).
53. Quarrie, S. *et al.* A monoclonal antibody to (S)-abscisic acid: its characterization and use in a radioimmunoassay for measuring abscisic acid in crude extracts of cereal and lupin leaves. *Planta* **173,** 330–339 (1988).
54. Sharp, R. & Davies, W. Variability among species in the apoplastic pH signalling response to drying soils. *J. Exp. Bot.* **60,** 4361–4370 (2009).
55. Zhang, J. & Davies, W. Does ABA in the xylem control the rate of leaf growth in soil-dried maize and sunflower plants? *J. Exp. Bot.* **41,** 1125–1132 (1990).
56. Ebel, R., Duan, X., Still, D. & Auge, R. Xylem sap abscisic acid concentration and stomatal conductance of mycorrhizal *Vigna unguiculata* in drying soil. *New Phytol.* **135,** 755–761 (1997).
57. Jones, M., Outlaw, W. & Lowry, O. Enzymic assay of 10^{-7} to 10^{-14} moles of sucrose in plant tissues. *Plant Physiol.* **60,** 379–383 (1977).
58. Ohshima, T., Hayashi, H. & Chino, M. Collection and chemical composition of pure phloem sap from *Zea mays* L. *Plant Cell Physiol.* **31,** 735–737 (1990).
59. Hayashi, H. & Chino, M. Chemical composition of phloem sap from the uppermost internode of the rice plant. *Plant Cell Physiol.* **31,** 247–251 (1990).
60. Nadwodnik, J. & Lohaus, G. Subcellular concentrations of sugar alcohols and sugars in relation to phloem translocation in *Plantago major*, *Plantago maritima*, *Prunus persica*, and *Apium graveolens*. *Planta* **227,** 1079–1089 (2008).
61. Leakey, A., Bernacchi, C., Ort, D. & Long, S. Long-term growth of soybean at elevated [CO_2] does not cause acclimation of stomatal conductance under fully open-air conditions. *Plant Cell Environ.* **29,** 1794–1800 (2006).
62. Long, S. P., Ainsworth, E. A., Rogers, A., Ort, D. R. Rising atmospheric carbon dioxide: plants FACE the future. *Annu. Rev. Plant Biol.* **55,** 591–628 (2004).

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Author contributions

S.B.G., E.A.A., C.J.B., S.P.L., D.R.O., D.M.R. and A.D.B.L. designed the research. S.B.G., O.D., S.P.K., A.M.L., J.M.M., R.E.P., D.M.R., U.M.R-V., M.H.S., R.S., E.A.A., C.J.B. and A.D.B.L. carried out field instrumentation, data collection and sample collection. S.B.G. carried out leaf and xylem biochemical/hormone analyses. S.P.K., R.E.P. and R.S. carried out root length measurements from minirhizotron images. S.B.G., D.M.R., U.M.R-V., E.A.A. and A.D.B.L. analysed data. S.B.G. and A.D.B.L. wrote the manuscript. All authors discussed the results and commented on the manuscript.

Additional information

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Competing interests

The authors declare no competing financial interests.