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Leaf and canopy scale drivers of genotypic variation in soybean response to elevated carbon dioxide concentration

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Abstract

The atmospheric [CO_2] in which crops grow today is greater than at any point in their domestication history and represents an opportunity for positive effects on seed yield that can counteract the negative effects of greater heat and drought this century. In order to maximize yields under future atmospheric [CO_2], we need to identify and study crop cultivars that respond most favorably to elevated [CO_2] and understand the mechanisms contributing to their responsiveness. Soybean (*Glycine max* Merr.) is a widely grown oilseed crop and shows genetic variation in response to elevated [CO_2]. However, few studies have studied the physiological basis for this variation. Here, we examined canopy light interception, photosynthesis, respiration and radiation use efficiency along with yield and yield parameters in two cultivars of soybean (Loda and HS93-4118) previously reported to have similar seed yield at ambient [CO_2], but contrasting responses to elevated [CO_2]. Seed yield increased by 26% at elevated [CO_2] (600 μmol/mol) in the responsive cultivar Loda, but only by 11% in HS93-4118. Canopy light interception and leaf area index were greater in HS93-4118 in ambient [CO_2], but increased more in response to elevated [CO_2] in Loda. Radiation use efficiency and harvest index were also greater in Loda than HS93-4118 at both ambient and elevated [CO_2]. Daily C assimilation was greater at elevated [CO_2] in both cultivars, while stomatal conductance was lower. Electron transport capacity was also greater in Loda than HS93-4118, but there was no difference in the response of photosynthetic traits to elevated [CO_2] in the two cultivars. Overall, this greater understanding of leaf- and canopy-level photosynthetic traits provides a strong conceptual basis for modeling genotypic variation in response to elevated [CO_2].

KEYWORDS

crop yield, elevated carbon dioxide concentration, free-air CO_2 enrichment, genotypic variation, *Glycine max*, photosynthesis, radiation use efficiency

1 | INTRODUCTION

Atmospheric carbon dioxide concentration ([CO_2]) is 44% higher in 2016 than it was in pre-industrial times (WMO, 2016). Over the past decade, [CO_2] has risen by an average 2.1 μmol mol^{-1} $year^{-1}$ and in 2015 the annual mean [CO_2] measured at Mauna Loa exceeded 400 μmol/mol for the first time in measured history (Betts, Jones, Knight, Keeling, & Kennedy, 2016). This increase in atmospheric

$[CO_2]$ is estimated to have contributed significantly to yield gains in C_3 crops over the past 30 years (McGrath & Lobell, 2013; Sakurai, Iizumi, Nishimori, & Yokozawa, 2014; Specht, Hume, & Kumudini, 1999; Specht et al., 2014). If emissions continue to grow at the current pace, $[CO_2]$ is expected to reach 500 μmol/mol by 2050 and 730–1,020 μmol/mol by 2100 (Ciais et al., 2013). In order to take advantage of the potential benefit from rising $[CO_2]$, it is important to identify C_3 crop cultivars that respond most favorably to elevated $[CO_2]$ and the mechanisms contributing to their responsiveness (Aspinwall et al., 2015).

Increasing $[CO_2]$ stimulates photosynthesis in C_3 crops by increasing the velocity of the Rubisco carboxylation reaction and decreasing the competitive oxygenation reaction that leads to photorespiration (Aranjuelo et al., 2013; Drake, González-Meler, & Long, 1997; Long, Ainsworth, Rogers, & Ort, 2004). Elevated $[CO_2]$ also decreases stomatal conductance (g_s) (Ainsworth & Rogers, 2007), which has the potential to scale to lower canopy transpiration and greater water use efficiency (Bernacchi & VanLoocke, 2015). These $[CO_2]$-induced responses typically improve C_3 crop biomass and yield at elevated $[CO_2]$ (Ainsworth & Long, 2005; Kimball, 1983; Kimball, Kobayashi, & Bindi, 2002; Long et al., 2004), although the magnitude and even the direction of the effect can vary with water availability and drought stress (Gray et al., 2016; Obermeier et al., 2017), nutrient availability (Long et al., 2004; Luo et al., 2004), and temperature (Ruiz-Vera et al., 2013). Soybean physiological and yield responses to elevated $[CO_2]$ have been broadly investigated in controlled environments (Ainsworth et al., 2002; Kumagai, Aoki, Masuya, & Shimono, 2015; Ziska, Bunce, & Caulfield, 1998, 2001) and in the field (Ainsworth, Rogers, Nelson, & Long, 2004; Bernacchi et al., 2006; Bishop, Betzelberger, Long, & Ainsworth, 2015; Bunce, 2015; Gray et al., 2016; Hao et al., 2012; Leakey et al., 2009; Morgan, Bollero, Nelson, Dohleman, & Long, 2005; Rogers et al., 2004, 2006). A consistent finding is that there is significant variation among soybean cultivars in their seed yield response to elevated $[CO_2]$ (Bishop et al., 2015; Bunce, 2015; Hao et al., 2012; Kumagai et al., 2015; Ziska et al., 1998, 2001). Variation in response has been attributed to increased branching or individual seed weight at elevated $[CO_2]$ (Ziska et al., 1998), delayed transition from vegetative to reproductive growth (Bunce, 2015), and plasticity in biomass accumulation, pod production, and partitioning (Bishop et al., 2015; Kumagai et al., 2015). While these studies potentially identify traits related to $[CO_2]$ response, they do not yet identify physiological mechanisms influencing these traits.

According to the Monteith (1977) formulation, yield potential (Y_p) can be calculated from photosynthetically active radiation (PAR) multiplied by the efficiencies by which light energy is intercepted by a crop canopy ($FPAR_i$), converted into biomass (RUE; radiation use efficiency), and partitioned to seeds (HI; harvest index). Dermody et al. (2008) reported that improvement in RUE was primarily responsible for the soybean yield response to elevated $[CO_2]$ in a commercial cultivar. In that study, $FPAR_i$ was not consistently stimulated by elevated $[CO_2]$ and HI was reduced by growth at elevated $[CO_2]$ (Morgan et al., 2005). Further study of 18 additional soybean cultivars revealed that HI was consistently lower in soybeans grown at elevated $[CO_2]$, and cultivars with greater HI tended to have greater seed yield response to elevated $[CO_2]$ (Bishop et al., 2015). Therefore, it is possible that genotypic variation in soybean yield responses to elevated $[CO_2]$ could be driven by changes in $FPAR_{i,}$ $RUE_,$ and HI. A recent meta-analysis showed RUE in soybean is positively correlated with atmospheric $[CO_2]$ in recent decades (Slattery & Ort, 2015), while a field study of historical soybean varieties found evidence that breeding has also resulted in improved RUE (Koester, Skoneczka, Cary, Diers, & Ainsworth, 2014). RUE is the slope of the relationship between the energy content of accumulated above-ground biomass vs. intercepted photosynthetically active radiation and generally represents the balance between photosynthesis and respiration (Sinclair & Muchow, 1999).

This 3-year field study investigates physiological mechanisms associated with the yield response of two soybean cultivars (HS93-4118 and Loda) previously shown to have very different yield responses to elevated $[CO_2]$ (Bishop et al., 2015). These two cultivars were among 18 cultivars initially tested for yield response to elevated $[CO_2]$, and they showed consistent differences in response to elevated $[CO_2]$ across four growing seasons, in contrast to other cultivars whose response was more variable (Bishop et al., 2015). Across 4 years of study, HS93-4118 showed no significant stimulation in yield at elevated $[CO_2]$ while Loda showed an average 24% stimulation in yield (Bishop et al., 2015). The two cultivars are similar in terms of maturity group and release date, have similar yields at ambient $[CO_2]$, and therefore provide a promising comparison for understanding the physiological basis for variation in yield response. Based on previous studies of a commercial genotype (Dermody et al., 2006), we predicted that the greater seed yield stimulation in Loda would be associated primarily with greater stimulation of RUE by elevated $[CO_2]$ linked with improved C balance of the leaf at elevated $[CO_2]$. We also hypothesized that Loda would have greater HI, supporting greater yields at elevated $[CO_2]$.

2 | MATERIALS AND METHODS

2.1 | Field site, experimental conditions, and soybean cultivars

This experiment was performed at the SoyFACE research facility (http://www.igb.illinois.edu/soyface/) in Champaign, IL, USA (40°02′N, 88°14′W; 228 m above sea level) during the 2012, 2013, and 2014 soybean growing seasons. This facility has been described previously by Ainsworth et al. (2004) and Rogers et al. (2004). The field experiment consisted of four blocks, each containing two 20 m diameter octagonal plots, with one control (i.e., ambient $[CO_2]$) and one elevated $[CO_2]$ plot (target $[CO_2]$ of 590–600 μmol/mol). Elevated $[CO_2]$ plots were fumigated during daylight hours using FACE technology (Miglietta et al., 2001), and the mean elevated $[CO_2]$ for each year is reported in Table 1. To avoid cross-contamination from the elevated plots to the ambient plots, rings were separated by 100 m (Miglietta et al., 2001). Weather conditions including air

TABLE 1 Meteorological conditions from planting to crop maturity, planting dates and average elevated [CO_2] during the 2012, 2013, and 2014 growing seasons at the SoyFACE experimental site in Champaign, IL

Year	Growing season precip (mm)	Ave air *T* (°C)	Total solar radiation (MJ/m^2)	Planting date	Harvest date	Elevated [CO_2] (μmol/mol)
2012	265.6	24.1	2,716	15-May	15-October	592.5 ± 1.2
2013	232.3	22.4	2,016	12-June	1-November	596.3 ± 13.6
2014	526.4	22.7	2,179	18-June	1-November	602.6 ± 6.6

temperature, relative humidity, solar radiation, and precipitation during growing season were recorded by an onsite weather station (Table 1; Fig. S1). The soil at the SoyFACE site is a Drummer-Flanagan soil, typical of central Illinois.

Two soybean cultivars (HS93-4118 and Loda) were planted with a row spacing of 0.38 m in the central part of each ring. In 2012 and 2013, each cultivar was planted over 4.56 m^2 (four rows of 3 m long), while in 2014 a larger area (16.41 m^2, eight rows of 5.4 m long) was planted per cultivar for sequential biomass harvesting during the growing season to calculate conversion efficiency (RUE). HS93-4118 is a maturity group II soybean that was released in 2000 with parent cultivars IA2007 and DSR304. Loda is also a maturity group II soybean released in 2000 with parent cultivars Jack and IA3003. Both varieties were obtained from the USDA soybean germplasm collection.

HS93-4118 and Loda were previously grown at SoyFACE and seed yield was measured at maturity each year from 2004 to 2008 (Fig. S2). The growing conditions and methods for measuring seed yield during those years are described by Bishop et al. (2015). These data were combined with the three years of data collected in the current study to provide 8 years of seed yield observations at ambient and elevated [CO_2].

2.2 | Crop growth, yield, and harvest index

During each growing season, developmental stage and yield traits were recorded using the two central rows of the cultivar's plots, meanwhile the two outside rows were used as borders. In 2013 and 2014, above-ground biomass (DM g/m), grain yield (tons/ha), HI (g seed per g aboveground biomass), and time to completion of maturity of 95% of the pods were measured. In 2012, grain yield was measured. When the cultivars reached maturity, yield was determined by harvesting 2 m of the two central rows from each plot with a small combine. For HI measurements in 2013 and 2014, 1 m of a row was harvested by hand, and the stems and pods were separated and dried at 60°C.

2.3 | Light interception, radiation use efficiency, sink:source ratio, and plant height

Measurements of canopy light interception were made once or twice per week throughout the 2014 growing season depending on the weather conditions. Photosynthetically active radiation (PAR) measurements were taken above (I_a) and below (I_b) the canopy in an undisturbed area (two central rows) of each plot during the middle of a clear day using a line quantum sensor (AccuPAR LP-80; Decagon Devices, Pullman, WA, USA), following the methods described in Koester et al. (2014). $FPAR_i$ was calculated as $1 - (I_a/I_b)$ (Nobel, Forseth, & Long, 1993). $FPAR_i$ weekly measurements stopped when most of the soybean foliage had senesced at the R7 developmental stage (Fehr, Caviness, Burmood, & Pennington, 1971). Mean $FPAR_i$ is the average of all weekly observations for each cultivar and CO_2 treatment up to R7.

During the 2014 growing season, aboveground biomass accumulation per unit area was measured every 10 days after the plants reached the V5 developmental stage (Fehr et al., 1971). Avoiding the borders of the plot, a 1 m section of row was harvested at ~2.5 cm above the ground. Plants were counted and

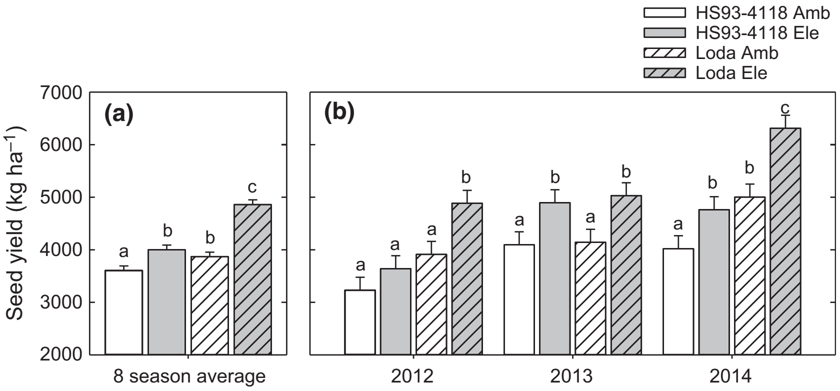

FIGURE 1 (a) Average seed yield measured over 8 growing seasons (2004–2008, 2012–2014) for HS93-4118 and Loda grown at ambient (Amb) and elevated (Ele) [CO_2]. Least square means and standard error of the least square means are plotted. (b) Average seed yield for the years of this study, 2012, 2013, and 2014. Different letters indicate statistically significant differences in least square mean values across the eight growing seasons (a) or within a given year (b) based on pair-wise comparisons of the means ($p < .05$)

separated into leaves, stems (including petioles and petiolules), and pods (when the plants reached reproductive stages). Plant material was dried at 60°C and then weighed. To convert the plant biomass into energy equivalents, leaves, stems, and seeds were ground and then analyzed separately for total energy content using an adiabatic bomb calorimeter (model 1261; Parr Instrument, Moline, IL, USA) with benzoic acid as standard. The cumulative intercepted radiation (PAR_i) at the time of each biomass harvest was calculated by multiplying the total PAR measured by the climatic station between biomass harvests, by the linearly interpolated $FPAR_i$. For calculation of seasonal RUE, cumulative biomass energy content from aboveground harvests (MJ/m^2) was regressed against PAR_i (MJ/m^2), and RUE was calculated from the slope of this linear relationship (Fig. S3; Monteith, 1972).

The sink:source ratio was estimated as the seed weight per plant divided by the leaf area per plant measured at canopy closure (Zhu et al., 2014). The leaf area of five plants per cultivar per plot was measured with a leaf area meter (LI-3000; LI-COR Biosciences, Lincoln, NE, USA). Plant height was measured in 2014 when the soybeans reached maturity. Five plants per plot were measured for each genotype and [CO_2] treatment and averaged for statistical analysis.

2.4 | Leaf area index and light extinction coefficient

Leaf area index (LAI) readings were taken approximately once per week throughout 2012, 2013, and 2014 growing season. Each measurement was taken in the evening to ensure diffuse light conditions (Hyer & Goetz, 2004) using a plant canopy analyzer (LAI2000; LI-COR Biosciences). An opaque mask with a 45° opening was used to restrict the viewing area of the lens. One reading above the canopy was taken immediately before and in the same direction as a set of four below canopy readings along a diagonal transect at 0%, 25%, 50%, and 75% of the distance across a row. One set of measurements were taken per cultivar per ring. The canopy light extinction coefficient (k) was estimated during and after canopy closure from transmitted (TPAR) and incoming PAR (PAR) according to the following equation (Flénet, Kiniry, Board, Westgate, & Reicosky, 1996):

$$k = -\ln(\mathrm{TPAR}/\mathrm{PAR})/\mathrm{LAI}.$$

2.5 | Gas exchange measurements

Diurnal measurements of leaf instantaneous photosynthetic CO_2 assimilation (A), and stomatal conductance (g_s), were measured using a portable infrared gas analyzer (Li-Cor 6400; LI-COR Biosciences). Measurements were made on the most recently fully expanded leaf at the top of the canopy during vegetative and reproductive developmental stages in 2012, 2013 and 2014. Gas exchange was measured every 2 hr from sunrise to sunset on three plants per cultivar per ring. Before each time of measurement, light intensity was recorded by a photosynthetic photon flux density meter (LI-190; LI-COR Biosciences) and temperature was monitored by the onsite

TABLE 2 Analysis of variance (F, p) of yield measured in 2012, 2013, and 2014, and yield determinants, stover, height, and source:sink ratio measured in 2014, for soybean cultivars HS93-4118 and Loda grown at ambient and elevated [CO_2]

	Seed yield	Interception efficiency ($FPAR_i$)	Radiation use efficiency (RUE)	Partitioning efficiency (HI)	Stover	Height	Source:Sink
[CO_2]	**$F_{1,6}$ = 25.6, .0028**	$F_{1,3}$ = 4.54, .1228	**$F_{1,3}$ = 6.43, .0433**	$F_{1,3}$ = 0.46, .5423	**$F_{1,3}$ = 6.42, .0446**	$F_{1,3}$ = 5.37, .0597	$F_{1,3}$ = 0.00, .9531
Cultivar (C)	**$F_{1,10}$ = 47.8, <.0001**	**$F_{1,6}$ = 78.7, .0001**	**$F_{1,6}$ = 47.8, .0010**	**$F_{1,6}$ = 11.3, .0396**	$F_{1,6}$ = 3.58, .1072	**$F_{1,6}$ = 59.5, .0045**	**$F_{1,6}$ = 10.3, .0184**
[CO_2] × C	**$F_{1,6}$ = 14.2, .0089**	**$F_{1,6}$ = 12.41, .0125**	$F_{1,6}$ = 0.23, .6534	$F_{1,6}$ = 0.86, .4196	$F_{1,6}$ = 0.70, .4354	$F_{1,6}$ = 0.03, .8584	$F_{1,6}$ = 0.38, .5603
Year	**$F_{7,44}$ = 18.7, <.0001**						
Year × [CO_2]	$F_{7,44}$ = 1.03, .4252						
Year × C	**$F_{7,37}$ = 5.10, .0004**						
Year × [CO_2] × C	$F_{7,37}$ = 0.73, .6468						

F's under scripted values correspond to numerator and denominator degrees of freedom for each term used in the ANOVA.

Bold indicates statistical significance (p < .05).

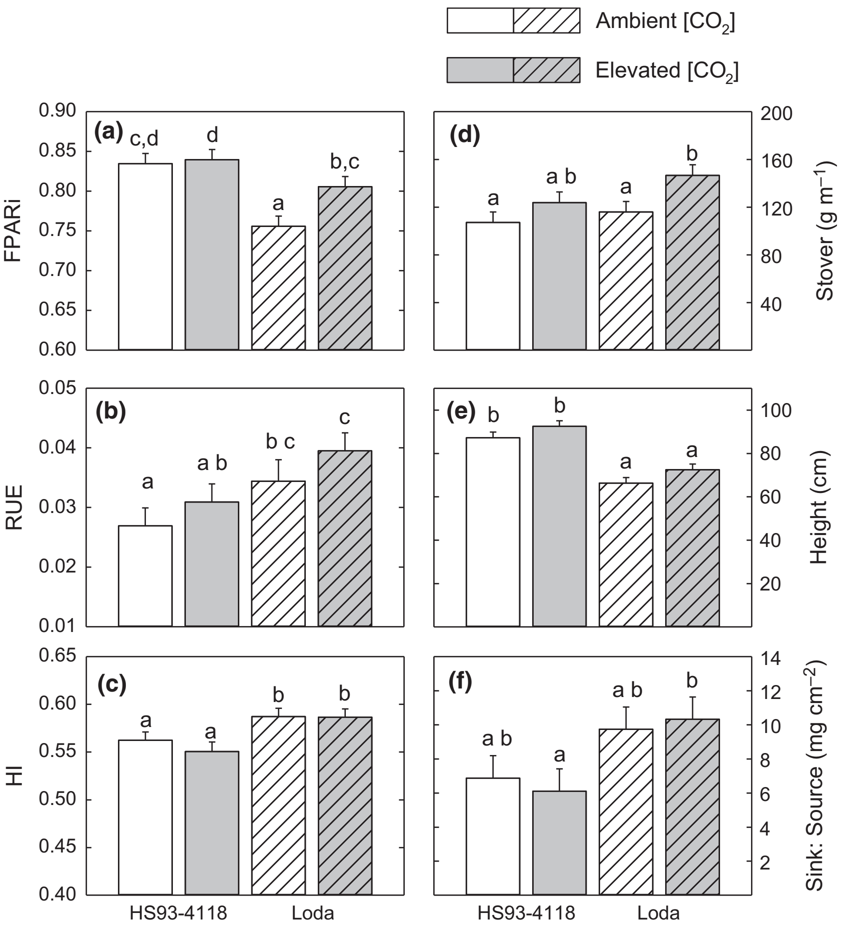

FIGURE 2 (a) Efficiency of canopy light interception (FPAR$_i$), (b) radiation use efficiency (RUE), (c) partitioning of biomass to seed or harvest index (HI), (d) end of season stem and leaf biomass (stover), (e) plant height (cm) measured at maturity, and (f) ratio of seed weight to maximum leaf area (source:sink) measured in HS93-4118 and Loda grown at ambient and elevated [CO$_2$] in 2014. Different letters indicate statistically significant differences in mean values based on pair-wise comparisons of the means ($p < .05$)

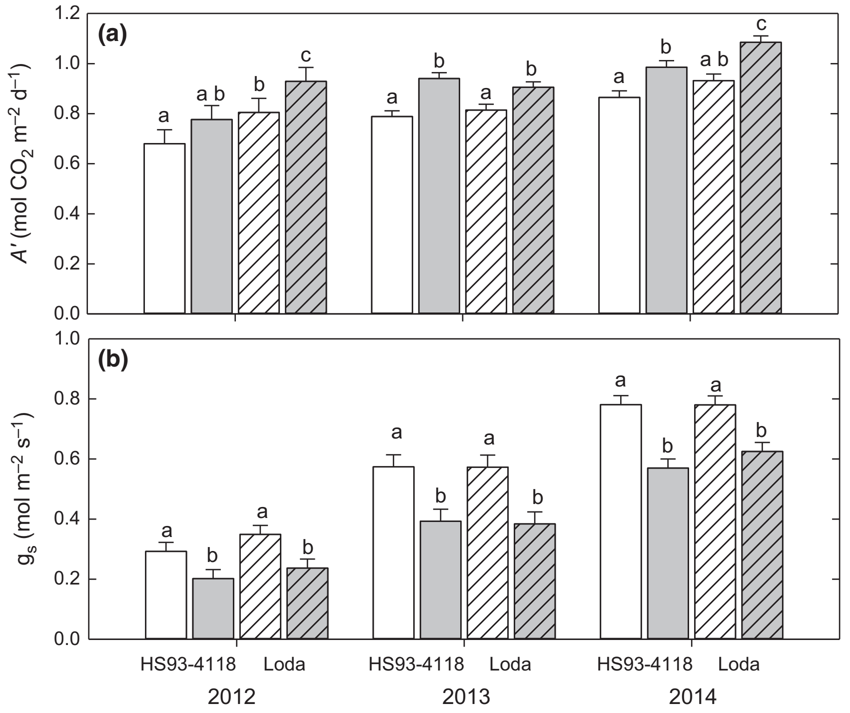

FIGURE 3 (a) Daily photosynthetic C gain (A'), and (b) midday stomatal conductance (g_s) measured in HS93-4118 and Loda grown at ambient and elevated [CO$_2$] in 2012, 2013, and 2014. The bar chart shows the seasonal average values, and diurnal photosynthesis was measured 3–4 times per growing season. Different letters indicate statistically significant differences in least square mean values within a given year based on pair-wise comparisons of the means ($p < .05$)

weather station. The conditions in the leaf cuvette were then set to match the ambient environmental conditions, with the [CO$_2$] in the cuvette set to match either ambient (~400 μmol/mol) or elevated [CO$_2$] treatment (590–600 μmol/mol). The total daily CO$_2$ uptake (A') was estimated by integrating under the area of the diurnal curve of photosynthesis.

TABLE 3 Analysis of variance (*F*, *p*) of diurnal C gain (A'), midday stomatal conductance (g_s), maximum Rubisco carboxylation rate ($V_{c,max}$), and RuBP regeneration rate (J_{max}) measured in 2012, 2013, and 2014 for HS93-4118 and Loda grown at ambient and elevated [CO_2]

	A' (mol CO_2 m^{-2} day^{-1})			g_s (mol m^{-2} s^{-1})			$V_{c,max}$ (μmol m^{-2} s^{-1})			J_{max} (μmol m^{-2} s^{-1})		
	2012	2013	2014	2012	2013	2014	2012	2013	2014	2012	2013	2014
CO_2	**6.97, .015**	**25.8, <.001**	**29.4, <.001**	**12.9, .002**	**29.4, <.001**	**34.8, <.001**	**36.2, <.001**	2.44, .128	1.43, .237	3.14, .085	0.39, .537	0.11, .741
Cultivar (C)	**11.0, .003**	0.41, .526	**10.9, .002**	2.61, .121	0.02, .884	0.74, .394	**5.27, .028**	**5.39, .026**	0.92, .41	**18.9, <.001**	**7.30, .012**	**6.24, .016**
G × CO_2	0.11, .746	0.24, .626	0.41, .526	0.14, .710	0.01, .906	0.79, .380	0.03, .865	1.02, .321	0.22, .639	0.14, .958	0.05, .827	0.28, .598
DOY	0.94, .343	**186, <.001**	**22.2, <.001**	**92.6, <.001**	**48.6, <.001**	**44.8, <.001**	**5.33, .009**	**20.5, <.001**	**20.7, <.001**	**23.1, <.001**	**10.4, <.001**	**7.63, <.001**
CO_2 × DOY	0.09, .773	1.54, .230	1.52, .223	**6.02, .023**	1.20, .316	2.60, .064	0.74, .485	0.37, .691	1.02, .393	2.11, .137	0.57, .569	0.65, .584
C × DOY	3.75, .066	0.38, .689	0.74, .536	4.13, .055	2.81, .075	2.67, .059	0.36, .70	2.50, .098	2.06, .119	0.26, .771	**3.89, .031**	2.17, .105
C × CO_2 × DOY	0.66, .425	0.12, .887	0.04, .990	0.33, .571	0.49, .671	0.32, .809	2.6, .089	1.19, .317	0.05, .985	0.98, .384	0.90, .418	0.37, .776

Parameters were measured on multiple days of year (DOY) across the growing season. Years were analyzed independently.

TABLE 4 Analysis of variance (*F*, *p*) of leaf N content and dark respiration (R_d) measured in HS93-4118 and Loda grown at ambient and elevated [CO_2]

	N (g/m^2)		R_d (μmol m^{-2} s^{-1})
	2013	2014	2014
[CO_2]	0.89, .3551	0.00, .9638	3.49, .0706
Cultivar (C)	4.19, .0533	2.25, .1483	2.07, .1592
[CO_2] × C	**4.91, .0379**	0.01, .9315	0.54, .4660
Day of year (DOY)	**15.31, .0008**	0.29, .5989	**46.1, <.0001**
DOY × [CO_2]	0.07, .7995	0.24, .6266	0.83, .4443
DOY × C	1.84, .1892	0.14, .7109	0.82, .4492
DOY × [CO_2] × C	**5.49, .0291**	1.94, .1782	0.12, .8877

Parameters were measured on multiple days of year (DOY) across the growing seasons. Years were analyzed independently.

In addition, the maximum rates of Rubisco carboxylation ($V_{c,max}$) and RuBP regeneration rate (J_{max}) were estimated from the response of *A* to intercellular [CO_2] (c_i), as previously described (Ainsworth et al., 2007). On the day after diurnal photosynthesis measurements, leaves were harvested predawn and kept at low light conditions prior to measurement to avoid transient decreases in water potential, decreases in chloroplast inorganic phosphate concentration, or decreases in maximum photosystem II efficiency (Bernacchi et al., 2005). To avoid breaking the xylem water column, petioles of the harvested leaves were immediately immersed in water, then re-cut again under water (Bernacchi et al., 2005). This approach of cutting leaves before dawn and measuring A/c_i response curves in a field laboratory enables measurements to be made with multiple leaf gas exchange systems at a common temperature and VPD. The approach has previously been shown to provide estimates of photosynthesis that are similar to those measured in the field (Ainsworth et al., 2004). In 2012, A/c_i curves were measured when the plants were in vegetative growth (V6, 2 July), beginning pod development (R3, 2 August), and beginning seed development (R5, 18 August) according to the growth stages defined by Fehr et al. (1971). In 2013, A/c_i curves were made at V6 (13 July), full bloom (R2, 27 July), and R5 (13 August). In 2014, A/c_i curves were made when plants were in vegetative growth (V5, 23 July), R2 (12 August), full pod (R4, 24 August), and full seed (R6, 7 September). Photosynthesis was initially induced at growth [CO_2] (ambient, 400 μmol/mol CO_2 or elevated, 600 μmol/mol CO_2). Next, [CO_2] was reduced stepwise to the lowest concentration of 50 μmol/mol and then increased stepwise to the highest CO_2 concentration of 1,500 μmol/mol. During the measurements, leaf temperature was ~25°C and PPFD was 1,500–1,750 μmol m^{-2} s^{-1}. $V_{c,max}$ and J_{max} were calculated using equations developed by Farquhar, von Caemmerer, and Berry (1980) with the temperature functions of Bernacchi, Singsaas, Pimentel, Portis, and Long (2001), Bernacchi, Pimentel, and Long (2003).

In 2014, dark adapted leaf respiration was measured as CO_2 efflux in situ from the youngest most fully expanded leaf at V5 (24 July), R4 (24 August), and R6 (7 September). Gas exchange measurements started 2 hr after sunset, and a larger leaf cuvette (2 × 6 cm) was used with the infrared gas analyzer (LI-6400) for measurements of CO_2 efflux. For each ring and cultivar, three leaves were measured. The block temperature of the Li-Cor 6400 was set according to the recorded ambient temperature, and the relative humidity was maintained between 60% and 70%.

2.6 | Leaf N content

In 2013, leaves were harvested for leaf N content at two growth stages, V6 and R5. In 2014, leaf disks were harvested at V5 and R4. At each time point, three 1.4 cm^2 disks from the middle leaflet of the youngest most fully expanded leaf from three different plants per plot were harvested and pooled. Disks were dried at 50°C, ground, weighed, and put into tin capsules. Leaf N content (g/m^2) was determined by dry combustion of the capsule in an elemental analyzer (Costech 4010; Thermo Scientific, Waltham, MA, USA).

2.7 | Statistical analysis

Seed yield was tested using a mixed model analysis of variance (PROC MIXED, SAS 9.4; SAS Institute, Cary, NC, USA) with growing season (year), [CO_2], and cultivar modeled as fixed effects, and block as a random effect. To test for significant differences between cultivars and [CO_2] treatments within a growing season, paired linear contrasts were done on the least squares means. Monteith parameters and sink:source were tested with a similar mixed model, except that growing season (year) was not included because parameters were only measured in 2014.

Leaf N and gas exchange parameters that were measured multiple times during a single growing season were tested with a repeated measures mixed model analysis of variance with the autoregressive covariance structure and Kenwood-Rogers specification for fixed effects and degrees of freedom. Growing seasons were analyzed independently. Cultivar and [CO_2] were fixed effects, the day of the year that the measurement was taken (DOY) was a repeated effect and block was a random effect in the model (PROC MIXED, SAS 9.4). When the main effect of [CO_2], cultivar, or DOY or their interaction were significant, paired linear contrasts were done on the least squares means to identify significant differences between cultivars and [CO_2] treatments.

The correlations and linear dependencies among yield, yield determinants, and photosynthetic parameters were tested with simple linear regression models (PROC CORR, PROC REG, SAS 9.4).

3 | RESULTS

3.1 | Yield and yield component responses to elevated [CO_2]

Averaged over eight growing seasons, the stimulation in seed yield at elevated [CO_2] was ~1,000 kg/ha (25.6%) in Loda and <400 kg/ha (10.9%) in HS93-4118 (Figure 1a), resulting in a significant cultivar × [CO_2] treatment interaction (Table 2). Loda had similar, but slightly greater yield in ambient [CO_2] as HS93-4118, and much greater yield in elevated [CO_2] (Figure 1a). This general pattern of similar yields at ambient [CO_2] and a greater yield responsiveness to elevated [CO_2] in Loda compared to HS93-4118 was consistent over eight growing seasons (Figure 1b; Fig. S1).

Yield determinants $FPAR_i$, RUE, and HI were measured in 2014. Loda had lower $FPAR_i$ and greater RUE than HS93-4118 (Figure 2a,b; Table 2). There was a trend toward greater $FPAR_i$

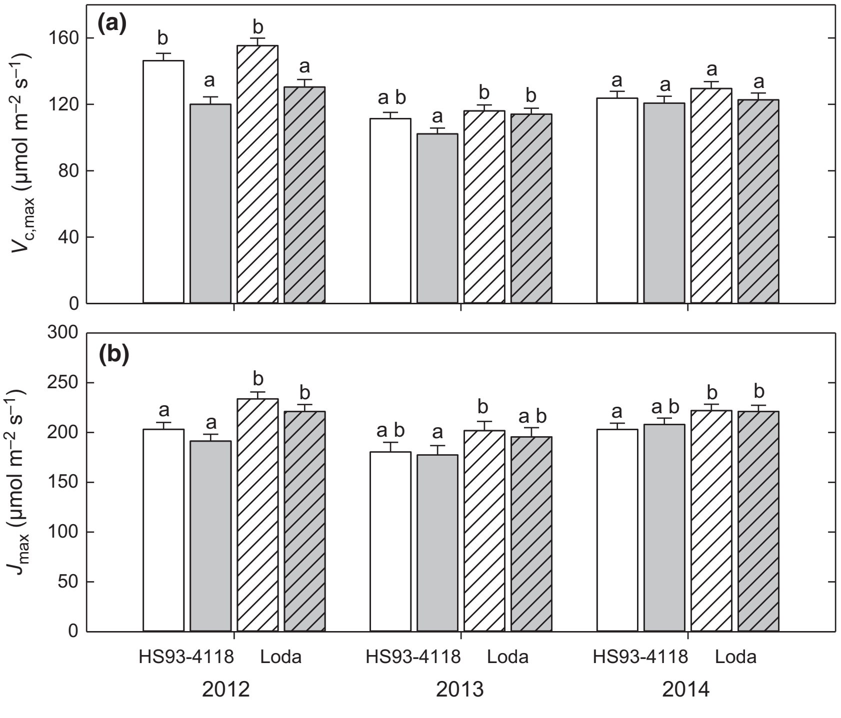

FIGURE 4 (a) Maximum rate of Rubisco carboxylation ($V_{c,max}$), and (b) RuBP regeneration rate (J_{max}) measured in HS93-4118 and Loda grown at ambient and elevated [CO_2] in 2012, 2013, and 2014. The bar chart shows the seasonal average values, and photosynthetic capacity was measured 3–4 times per growing season. Different letters indicate statistically significant differences in least square mean values within a given year based on pairwise comparisons of the means ($p < .05$)

and RUE in elevated [CO_2] (Figure 2a,b; Table 2). The FPAR$_i$ results were consistent with LAI measurements, which were taken in 2012, 2013, and 2014 (Fig. S4). HS93-4118 had greater peak LAI than Loda, and more rapid canopy closure especially in elevated [CO_2] (Fig. S4). This allowed for greater early season FPARi in HS93-4118 compared to Loda and slightly greater light extinction coefficients (Fig. S5).

There was no significant effect of [CO_2] on HI in 2014, but Loda had significantly greater HI than HS93-4118 (Figure 2c; Table 2). End of season stem and leaf biomass (stover) was increased by elevated [CO_2], although the pairwise comparison of means showed it was only statistically significant for Loda (Figure 2d; Table 2). Loda was significantly shorter than HS93-4118, and height was not significantly affected by growth at elevated [CO_2] in either cultivar (Figure 2e; Table 2). The ratio of carbon sinks (seed mass) to sources (peak leaf area) tended to be greater in Loda compared to HS93-4118 (Figure 2f), however, elevated [CO_2] did not alter this parameter.

3.2 | Leaf photosynthetic and respiration responses to elevated [CO_2]

Diurnal measurements of photosynthesis were made during the 2012, 2013, and 2014 growing seasons. By integrating under the diurnal curve, daily photosynthetic C gain (A') was estimated. A' was consistently greater in elevated [CO_2] in both cultivars (Figure 3a; Table 3), and Loda had greater A' than HS93-4118 when averaged across [CO_2] treatments in 2012 and 2014 (Figure 3a; Table 3). Both cultivars also showed reductions in midday stomatal conductance (g_s) at elevated [CO_2] consistently across growing seasons (Figure 3b; Table 3). $V_{c,max}$ was down-regulated in elevated [CO_2] in both cultivars in 2012, but not in 2013 or 2014 (Figure 4a). There was no evidence for down-regulation of J_{max} in any year or cultivar (Figure 4b; Table 3). Supporting greater daily C assimilation, Loda had greater photosynthetic capacity than HS93-4118, especially J_{max} (Figure 4b; Table 3).

Dark respiration was measured in the 2014 growing season on three different dates corresponding to different developmental stages. Respiration rates tended to be greater at elevated [CO_2] ($p < .10$; Figure 5a), but were not significantly different between cultivars (Figure 5a; Table 3). Leaf N content was not consistently affected by elevated [CO_2] in 2013 and 2014 (Figure 5b,c; Table 3). In 2013, leaf N was significantly greater in Loda grown at ambient [CO_2] than Loda grown at elevated [CO_2] and greater than HS93-4118 (Figure 5b), but in 2014, there were no significant effects of [CO_2] or cultivar on leaf N (Figure 5c, Table 3).

3.3 | Correlations between seed yield, photosynthetic parameters, and yield determinants

Using data from 2014, the relationship between leaf physiological traits averaged over the growing season and RUE was explored with simple correlation analysis (Figure 6). Theoretically, greater photosynthetic rates support greater RUE (Monteith, 1977; Reynolds, van Ginkel, & Ribaut, 2000; Sinclair & Muchow, 1999), and in this experiment across genotypes and [CO_2] treatments, RUE was positively correlated with A' (Figure 6). RUE was also positively correlated with R_d (Figure 6), and weakly negatively correlated with $V_{c,max}$. A' was positively correlated with J_{max} and R_d, but not correlated with $V_{c,max}$ (Figure 6). There were no significant correlations among $V_{c,max}$, J_{max}, and R_d when averaged across the growing season (Figure 6).

Soybean seed yield was correlated with yield determinants, FPARi, RUE, and HI, and the relationship was altered by growth elevated [CO_2] (Figure 7). In both ambient and elevated [CO_2], there was a negative

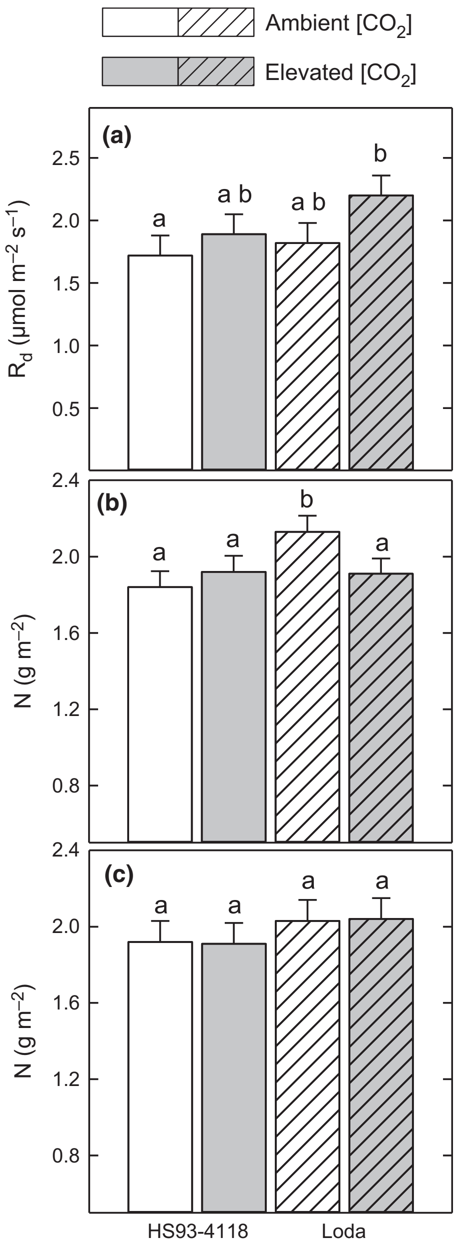

FIGURE 5 (a) Dark respiration (R_d) measured in 2014 and leaf N content measured in 2013 (b) and 2014 (c) for HS93-4118 and Loda grown at ambient and elevated [CO_2]. The bar chart shows the seasonal average values from multiple measurements. Different letters indicate statistically significant differences in least square mean values within a given year based on pair-wise comparisons of the means ($p < .05$)

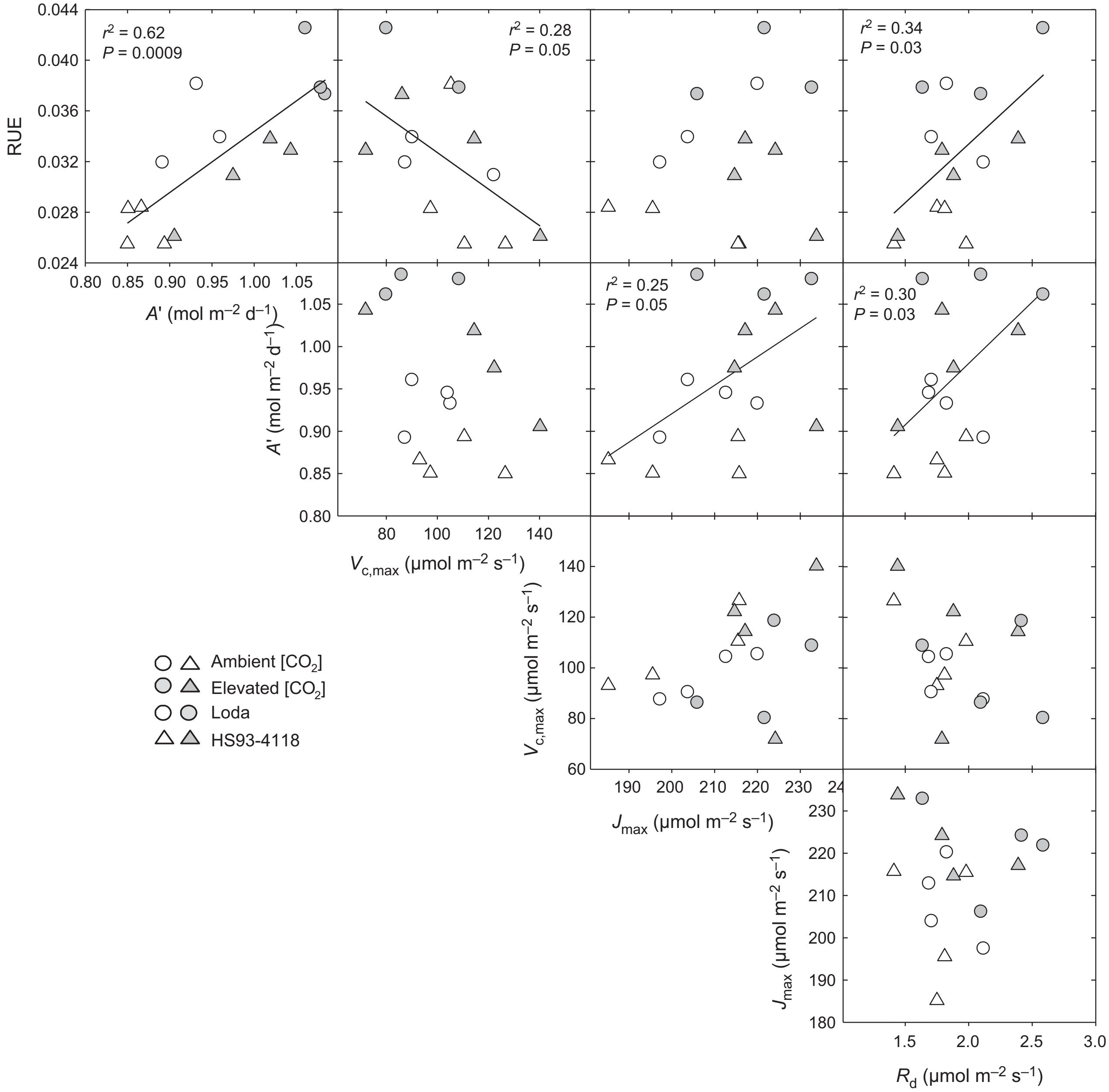

FIGURE 6 Correlations between radiation use efficiency (RUE), daily C gain (A'), maximum rate of Rubisco carboxylation ($V_{c,max}$), maximum RuBP regeneration rate (J_{max}), and dark respiration rate (R_d). Each symbol represents the seasonal mean value for each replicate in ambient and elevated [CO$_2$] per genotype (n = 4). Significant correlations are indicated by solid lines

linear correlation between yield and FPARi (Figure 7). This negative relationship was driven by HS93-4118's taller canopy with greater LAI and FPARi, but lower seed yields compared to Loda. Seed yield was positively correlated with RUE in elevated [CO$_2$] and positively correlated with HI in both ambient and elevated [CO$_2$] (Figure 7).

4 | DISCUSSION

This study investigated the physiological basis for variation in soybean response to elevated [CO$_2$] by comparing two cultivars with similar release dates and maturity groups, but contrasting yield responses to elevated [CO$_2$] (Bishop et al., 2015). Growth at elevated [CO$_2$] consistently increased seed yield in Loda across eight growing seasons, with an average increase of 25.6%. In contrast, seed yield was not stimulated in HS93-4118 at elevated [CO$_2$] in five of the eight growing seasons (Figure 1; Fig. S2). Previous research at SoyFACE indicated that greater RUE at elevated [CO$_2$] contributed more to the improvements in seed yield than changes in FPARi or HI (Dermody et al., 2008; Morgan et al., 2005; Zhu, Long, & Ort, 2010). However, in this study, growth at elevated [CO$_2$] increased RUE in both HS93-4118 and Loda, with no interaction of cultivar and treatment (Table 2). Instead, we found evidence that plasticity in FPAR$_i$ contributed to the greater yield response. Loda

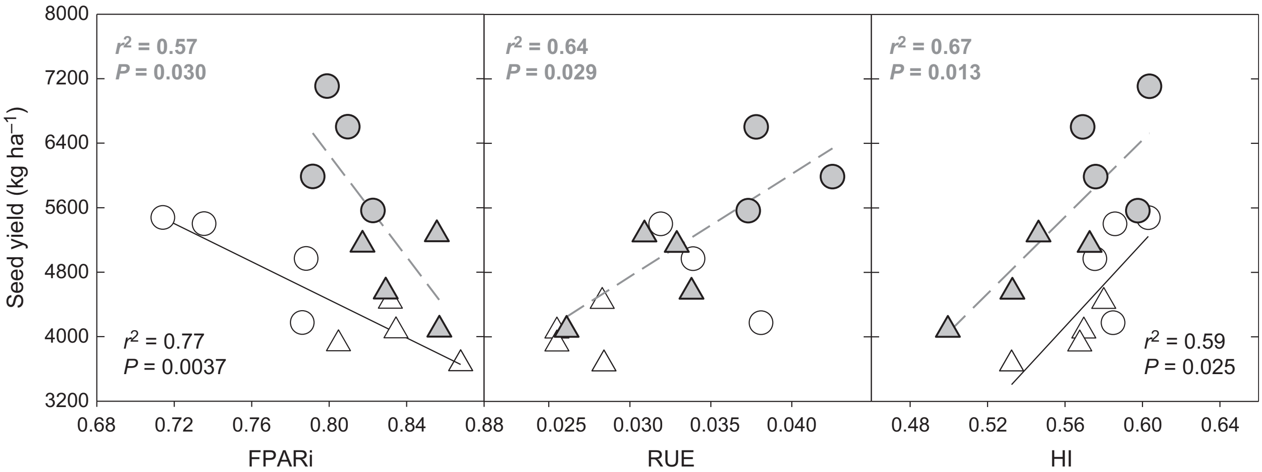

FIGURE 7 Correlations between seed yield and the efficiency of canopy light interception (FPAR$_i$), radiation use efficiency (RUE), and biomass partitioning to seed (HI) measured in 2014. Each symbol represents one replicate per genotype and treatment (n = 4). Significant correlations between variables are indicated for ambient [CO$_2$] (black lines) and elevated [CO$_2$] (gray dashed lines). Symbols are defined in Figure 6

had a smaller canopy than HS93-4118, and showed a small, but significant increase in FPAR$_i$ at elevated [CO$_2$], while there was no change in FPAR$_i$ in HS93-4118. This resulted in a significant [CO$_2$] × cultivar interaction for FPAR$_i$ (Table 2). HI was 5% greater in Loda than HS93-4118, and not significantly affected by elevated [CO$_2$]. This was a surprising result given that both of these cultivars and many others showed consistent reductions in HI when grown at elevated [CO$_2$] in previous seasons at SoyFACE (Bishop et al., 2015; Morgan et al., 2005). However, in 2014 the crop was planted late (June 18) which may have contributed to high HI (Unkovich, Baldock, & Forbes, 2010), and the growing conditions were excellent with abundant rainfall and moderate temperatures (Fig. S1), which may explain the high overall yields.

The combination of lower LAI and FPAR$_i$ and greater HI resulted in greater sink:source ratio in Loda compared to HS93-4118 (Figure 2e). In rice, sink:source ratio increased in a [CO$_2$] responsive cultivar and decreased in a less responsive cultivar (Zhu et al., 2014) and a large reproductive sink was needed for maximal CO$_2$ response (Hasegawa et al., 2013). But in this study with soybean, sink:source ratio did not change with growth at elevated [CO$_2$] in either cultivar. Soybean canopies have very high LAI, and it has been argued that current dense soybean canopies are not optimal for productivity or resource use efficiency at current or elevated [CO$_2$] (Drewry, Kumar, & Long, 2014; Srinivasan, Kumar, & Long, 2016). It has also been argued that FPAR$_i$ is close to its theoretical maximum, so perhaps there is little room for improvement (Zhu et al., 2010). In this study, both cultivars showed increased LAI at elevated [CO$_2$], while only Loda, the yield-responsive cultivar with lower LAI, also showed a significant increase in FPAR$_i$ at elevated [CO$_2$]. The fact that LAI can increase at elevated [CO$_2$] with no impact on FPAR$_i$ and the negative correlation between FPAR$_i$ and seed yield within a [CO$_2$] treatment (Figure 7) appears to support the hypothesis that LAI is higher than optimal for modern soybean cultivars (Srinivasan et al., 2016).

In theory and practice, RUE is dependent upon leaf and canopy CO$_2$ exchange rates, determined by both leaf photosynthetic CO$_2$ assimilation and mitochondrial respiration (Sinclair & Horie, 1989; Sinclair & Muchow, 1999). Although canopy photosynthesis was not measured in this study, diurnal photosynthetic measurements were made over three years and multiple growth stages, and we found that average seasonal A' was significantly correlated to RUE (Figure 6). Both cultivars showed significantly greater A' at elevated [CO$_2$], and in 2012 and 2014, Loda had significantly greater A' on average across the growing seasons than HS93-4118 (Table 2). Loda also had greater photosynthetic capacity than HS93-4118, especially J_{max}. Thus, greater photosynthetic capacity in Loda compared to HS93-4118 likely contributed to higher seed yields in both ambient and elevated [CO$_2$]. Bernacchi et al. (2013) previously modeled that increasing J_{max} improved photosynthesis in soybean under both ambient and elevated [CO$_2$], theoretically supporting our observation that J_{max} was greater in the higher yielding cultivar and positively correlated to A' (Figure 6). A significant correlation between photosynthetic C assimilation rate and yield in ambient and elevated [CO$_2$] has previously been reported for rice (De Costa, Weerakoon, Chinthaka, Herath, & Abeywardena, 2007), but our previous measurements of midday photosynthesis on six soybean cultivars did not show any significant correlation with yield (Bishop et al., 2015). That previous analysis was limited to midday measurements which most commonly represent light-saturated photosynthetic values, and was also limited to a single measurement in the growing season, which missed the seed filling period when canopy photosynthesis has been reported to be most strongly correlated to soybean yield (Kumudini, 2002).

Another component that could affect yield through RUE is R_d. R_d tended to increase at elevated [CO$_2$] as has been previously reported for soybean (Davey et al., 2004; Leakey et al., 2009) and other crops (e.g., Griffin & Turnbull, 2013). This was true for both cultivars, and the increase in respiration at elevated [CO_2] tended to be greater for Loda (+19%) than for HS93-4118 (+8%), resulting in a significant correlation between RUE and R_d (Figure 6). Nutrient or N availability can also impact RUE (Sinclair & Muchow, 1999) and its response to elevated [CO_2] (Slattery, Ainsworth, & Ort, 2013). However, in 2014 when RUE was measured, there were no significant differences in leaf N concentration due to elevated [CO_2] or cultivar. Hence, leaf N concentration did not appear to play a direct role in the variation in RUE response to elevated [CO_2] in HS93-4118 and Loda.

This study tested the hypothesis that greater photosynthesis and RUE would be associated with genetic variation in soybean response to elevated [CO_2]. Across multiple field seasons, Loda showed nearly double the stimulation in yield at elevated [CO_2] as HS93-4118. RUE and HI were significantly higher in Loda than HS93-4118 in both ambient and elevated [CO_2], but greater plasticity in the response of $FPAR_i$ likely contributed to a greater seed yield response to elevated [CO_2]. Photosynthetic capacity was also greater in Loda than HS93-4118, supporting the hypothesis that increasing A' could drive greater productivity in both current and future [CO_2]. Previous research with a commercial soybean cultivar showed that the expected stimulation in yield at elevated [CO_2] could be decreased and even reversed with increasing drought stress (Gray et al., 2016). Here, we demonstrate a consistent stimulation in yield at elevated [CO_2] in the soybean cultivar Loda across eight growing seasons with variable temperature and drought stress emphasizing that cultivar selection will be a key component of effective adaptation to global change.

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REFERENCES

Ainsworth, E. A., Davey, P. A., Bernacchi, C. J., Dermody, O. C., Heaton, E. A., Moore, D. J. . . . Long, S. P. (2002). A meta-analysis of elevated [CO_2] effects on soybean (*Glycine max*) physiology, growth and yield. *Global Change Biology*, *8*, 695–709.

Ainsworth, E. A., & Long, S. P. (2005). 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 Phytologist*, *165*, 351–372.

Ainsworth, E. A., & Rogers, A. (2007). The response of photosynthesis and stomatal conductance to rising [CO_2]: Mechanisms and environmental interactions. *Plant, Cell and Environment*, *30*, 258–270.

Ainsworth, E. A., Rogers, A., Leakey, A. D. B., Heady, L. E., Gibon, Y., Stitt, M., & Schurr, U. (2007). Does elevated atmospheric [CO_2] alter diurnal C uptake and the balance of C and N metabolites in growing and fully expanded soybean leaves? *Journal of Experimental Botany*, *58*, 579–591.

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

Aranjuelo, I., Sanz-Saez, A., Jauregui, I., Irigoyen, J. J., Araus, J. L., Sanchez-Diaz, M., & Erice, G. (2013). Harvest index, a parameter conditioning responsiveness of wheat plants to elevated CO_2. *Journal of Experimental Botany*, *64*, 1879–1892.

Aspinwall, M. J., Loik, M. E., Resco de Dios, V., Tjoelker, M. G., Payton, P. R., & Tissue, D. T. (2015). Utilizing intraspecific variation in phenotypic plasticity to bolster agricultural and forest productivity under climate change. *Plant, Cell and Environment*, *38*, 1752–1764.

Bernacchi, C. J., Bagley, J. E., Serbin, S. P., Ruiz-Vera, U. M., Rosenthal, D. M., & VanLoocke, A. (2013). Modelling C_3 photosynthesis from the chloroplast to the ecosystem. *Plant, Cell and Environment*, *36*, 1641–1657.

Bernacchi, C. J., Leakey, A. D. B., Heady, L. E., et al. (2006). 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 and Environment*, *29*, 2077–2090.

Bernacchi, C. J., Morgan, P. B., Ort, D. R., & Long, S. P. (2005) The growth of soybean under free air [CO_2] enrichment (FACE) stimulates photosynthesis while decreasing in vivo Rubisco capacity. *Planta*, *220*, 424–446.

Bernacchi, C. J., Pimentel, C., & Long, S. P. (2003). *In vivo* temperature response functions of parameters required to model RuBP-limited photosynthesis. *Plant, Cell and Environment*, *26*, 1419–1430.

Bernacchi, C. J., Singsaas, E. L., Pimentel, C., Portis, A. R., & Long, S. P. (2001). Improved temperature response functions for models of Rubisco-limited photosynthesis. *Plant, Cell and Environment*, *24*, 253–259.

Bernacchi, C. J., & VanLoocke, A. (2015). Terrestrial ecosystems in a changing environment: A dominant role for water. *Annual Review of Plant Biology*, *66*, 599–622.

Betts, R. A., Jones, C. D., Knight, J. R., Keeling, R. F., & Kennedy, J. J. (2016). El Nino and a record CO_2 rise. *Nature Climate Change*, *6*, 806–810.

Bishop, K. A., Betzelberger, A. M., Long, S. P., & Ainsworth, E. A. (2015). 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 and Environment*, *38*, 1765–1774.

Bunce, J. A. (2015). Elevated carbon dioxide effects on reproductive phenology and seed yield among soybean cultivars. *Crop Science*, *55*, 339–343.

Ciais, P., Sabine, C., Bala, G., Bopp, L., Brovkin, V., Canadell, J., & Thornton, P. (2013). Carbon and other biogeochemical cycles. In T. F. Stocker, D. Qin, G.-K. Plattner, M. Tignor, S. K. Allen, J. Boschung, . . . P. M. Midgley (Eds.), *Climate change 2013: The physical science basis. Contribution of working group I to the fifth assessment report of the intergovernmental panel on climate change* (pp. 465–570). Cambridge, UK and New York, NY: Cambridge University Press.

Davey, P. A., Hunt, S., Hymus, G. J., DeLucia, E. H., Drake, B. G., Karnosky, D. F. . . . Long, S. P. (2004). Greater oxygen uptake is not decreased by an instantaneous elevation of [CO_2], but is increased with long-term growth in the field at elevated [CO_2]. *Plant Physiology*, *134*, 520–527.

De Costa, W. A. J. M., Weerakoon, W. M. W., Chinthaka, K. G. R., Herath, H. M. L. K., & Abeywardena, R. M. I. (2007). Genotypic variation in the response of rice (*Oryza sativa* L.) to increased atmospheric carbon dioxide and its physiological basis. *Journal of Agronomy & Crop Science*, *193*, 117–130.

Dermody, O., Long, S. P., McConnaughay, K., & DeLucia, E. H. (2008). How do elevated CO_2 and O_3 affect the interception and utilization of radiation by a soybean canopy? *Global Change Biology*, *14*, 556–564.

Drake, B. G., González-Meler, M. A., & Long, S. P. (1997). More efficient plants: A consequence of rising atmospheric CO_2. *Annual Review of Plant Physiology and Plant Molecular Biology*, *48*, 609–639.

Drewry, D. T., Kumar, P., & Long, S. P. (2014). Simultaneous improvement in productivity, water use, and albedo through crop structural modification. *Global Change Biology*, *20*, 1955–1967.

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

Fehr, W. R., Caviness, C. E., Burmood, D. T., & Pennington, J. S. (1971). Stage of development descriptions for soybeans, *Glycine max* (L.) Merr. *Crop Science*, *11*, 929–931.

Flénet, F., Kiniry, J. R., Board, J. E., Westgate, M. E., & Reicosky, D. C. (1996). Row spacing effects on light extinction coefficients of corn, sorghum, soybean and sunflower. *Agronomy Journal*, *88*, 185–190.

Gray, S. B., Siebers, M., Locke, A. M., et al. (2016). Intensifying drought eliminates the expected benefits of elevated [CO_2] for soybean. *Nature Plants*, *2*, 16132.

Griffin, K. L., & Turnbull, M. H. (2013). Light saturated RuBP oxygenation by Rubisco is a robust predictor of light inhibition of respiration in *Tritcum aestivum* L. *Plant Biology*, *15*, 769–775.

Hao, X. Y., Han, X., Lam, S. K., Wheeler, T., Ju, H., Wang, H. R., . . . Lin, E. D. (2012). Effects of fully open-air [CO_2] elevation on leaf ultrastructure, photosynthesis, and yield of two soybean cultivars. *Photosynthetica*, *50*, 362–370.

Hasegawa, T., Sakai, H., Tokida, T., et al. (2013). Rice cultivar responses to elevated CO_2 at two free-air CO_2 enrichment (FACE) sites in Japan. *Functional Plant Biology*, *40*, 148–159.

Hyer, E. J., & Goetz, S. J. (2004). Comparison and sensitivity analysis of instruments and radiometric methods for LAI estimation: Assessments from a boreal forest site. *Agricultural and Forest Meteorology*, *122*, 157–174.

Kimball, B. A. (1983). Carbon dioxide and agricultural yield – An assemblage and analysis of 430 prior observations. *Agronomy Journal*, *75*, 779–788.

Kimball, B. A., Kobayashi, K., & Bindi, M. (2002). Responses of agricultural crops to free-air CO_2 enrichment. *Advances in Agronomy*, *77*, 293–368.

Koester, R. P., Skoneczka, J. A., Cary, T. R., Diers, B. W., & Ainsworth, E. A. (2014). Historical gains in soybean (*Glycine max* Merr.) seed yield are driven by linear increases in light interception, energy conversion, and partitioning efficiencies. *Journal of Experimental Botany*, *65*, 3311–3321.

Kumagai, E., Aoki, N., Masuya, Y., & Shimono, H. (2015). Phenotypic plasticity conditions the response of soybean seed yield to elevated atmospheric CO_2 concentration. *Plant Physiology*, *169*, 2021–2029.

Kumudini, S. (2002). Trials and tribulations: A review of the role of assimilate supply in soybean genetic yield improvement. *Field Crops Research*, *75*, 211–222.

Leakey, A. D. B., Xu, F., Gillespie, K. M., McGrath, J. M., Ainsworth, E. A., & Ort, D. R. (2009). Genomic basis for stimulated respiration by plants growing under elevated carbon dioxide. *Proceedings of the National Academy of Sciences of the United States of America*, *106*, 3597–3602.

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

Luo, Y., Su, B., Currie, W. S., et al. (2004). Progressive nitrogen limitation of ecosystem responses to rising atmospheric carbon dioxide. *BioScience*, *54*, 731–739.

McGrath, J. M., & Lobell, D. B. (2013). Regional disparities in the CO_2 fertilization effect and implications for crop yields. *Environmental Research Letters*, *8*, 014054.

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

Monteith, J. L. (1972). Solar radiation and productivity in tropical ecosystems. *Journal of Applied Ecology*, *9*, 747–766.

Monteith, J. L. (1977). Climate and the efficiency of crop production in Britain. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, *281*, 277–294.

Morgan, P. B., Bollero, G. A., Nelson, R. L., Dohleman, F. G., & Long, S. P. (2005). Smaller than predicted increase in aboveground net primary production and yield of field-grown soybean under fully open-air [CO_2] elevation. *Global Change Biology*, *11*, 1856–1865.

Nobel, P. S., Forseth, I. N., & Long, S. P. (1993). Canopy structure and light interception. In D. O. Hall, J. M. O. Scurlock, H. R. Bolhar-Nordenkampf, R. C. Leegood & S. P. Long (Eds.), *Photosynthesis and production in a changing climate* (pp. 79–90). London, UK: Chapman and Hall.

Obermeier, W. A., Lehnert, L. W., Kammann, C. I., Müller, C., Grünhage, L., Luterbacher, J., . . . Bendix, J. (2017). Reduced CO_2 fertilization effect in temperature C_3 grasslands under more extreme weather conditions. *Nature Climate Change*, *7*, 131–141.

Reynolds, M. P., van Ginkel, M., & Ribaut, J.-M. (2000). Avenues for genetic modification of radiation use efficiency in wheat. *Journal of Experimental Botany*, *51*, 459–473.

Rogers, A., Allen, D. J., Davey, P. A., et al. (2004). Leaf photosynthesis and carbohydrate dynamics of soybeans grown throughout their lifecycle under free-air carbon dioxide enrichment. *Plant, Cell and Environment*, *27*, 449–458.

Rogers, A., Gibon, Y., Stitt, M., Morgan, P. B., Bernacchi, C. J., Ort, D. R., & Long, S. P. (2006). Increased C availability at elevated carbon dioxide concentration improves N assimilation in a legume. *Plant, Cell and Environment*, *29*, 1651–1658.

Ruiz-Vera, U. M., Siebers, M., Gray, S. B., Drag, D. W., Rosenthal, D. M., Kimball, B. A., . . . Bernacchi, C. J. (2013). Global warming can negate the expected CO_2 stimulation in photosynthesis and productivity for soybean grown in the Midwestern United States. *Plant Physiology*, *162*, 410–423.

Sakurai, G., Iizumi, T., Nishimori, M., & Yokozawa, M. (2014). How much has the increase in atmospheric CO_2 directly affected past soybean production? *Nature Scientific Reports*, *4*, 1–5.

Sinclair, T. R., & Horie, T. (1989). Leaf nitrogen, photosynthesis, and crop radiation use efficiency: A review. *Crop Science*, *29*, 90–98.

Sinclair, T. R., & Muchow, R. C. (1999). Radiation use efficiency. *Advances in Agronomy*, *65*, 215–265.

Slattery, R. A., Ainsworth, E. A., & Ort, D. R. (2013). A meta-analysis of responses of canopy photosynthetic conversion efficiency to environmental factors reveals major causes of yield gap. *Journal of Experimental Botany*, *64*, 3723–3733.

Slattery, R. A., & Ort, D. R. (2015). Photosynthetic energy conversion efficiency: Setting a baseline for gauging future improvements in important food and biofuel crops. *Plant Physiology*, *168*, 383–392.

Specht, J. E., Diers, B. W., Nelson, R. L., Toledo, F. J., Torrion, J. A., & Grassini, P. (2014) Soybean. In S. Smith, B. Diers, J. Specht & B. Carver (Eds.), *Yield gains in major U.S. field crop* (pp. 311–356). Madison, WI: CSSA Special Publication 33.

Specht, J. D., Hume, J. D., & Kumudini, S. V. (1999). Soybean yield potential—A genetic and physiological perspective. *Crop Science*, *39*, 1560–1570.

Srinivasan, V., Kumar, P., & Long, S. P. (2016). Decreasing, not increasing, leaf area will raise crop yields under global atmospheric change. *Global Change Biology*, *23*, 1626–1635. doi: 10.1111/gcb.13526

Unkovich, M., Baldock, J., & Forbes, M. (2010). Variability in harvest index of grain crops and potential significance for carbon accounting: Examples from Australian agriculture. *Advances in Agronomy*, *105*, 173–219.

WMO (2016) *WMO Greenhouse Gas Bulletin: The State of Greenhouse Gases in the Atmosphere Based on Global Observations through 2015*. Bulletin No. 12, 1–8.

Zhu, X. G., Long, S. P., & Ort, D. R. (2010). Improving photosynthetic efficiency for greater yield. *Annual Review of Plant Biology*, *61*, 235–261.

Zhu, C., Zhu, J., Cao, J., Jiang, Q., Liu, G., & Ziska, L. H. (2014). Biochemical and molecular characteristics of leaf photosynthesis and relative seed yield of two contrasting rice cultivars in response to elevated [CO_2]. *Journal of Experimental Botany*, *65*, 6049–6056.

Ziska, L. H., Bunce, J. A., & Caulfield, F. (1998). Intraspecific variation in seed yield of soybean (*Glycine max*) in response to increased atmospheric carbon dioxide. *Australian Journal of Plant Physiology*, *25*, 801–807.

Ziska, L. H., Bunce, J. A., & Caulfield, F. A. (2001). Rising atmospheric carbon dioxide and seed yield of soybean genotypes. *Crop Science*, *41*, 385–391.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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