

Cassava about-FACE: Greater than expected yield stimulation of cassava (*Manihot esculenta*) by future CO₂ levels

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Abstract

Globally, cassava is the second most important root crop after potatoes and the fifth most important crop overall in terms of human caloric intake. In addition to its growing global importance for feed, fuel, and starch, cassava has long been vital to food security in Sub-Saharan Africa. Climate change is expected to have its most severe impact on crops in food insecure regions, yet little is known about how cassava productivity will respond to climate change. The most important driver of climate change is globally increasing atmospheric CO₂ concentration ([CO₂]). However, the potential for cassava to enhance food security in an elevated [CO₂] world is uncertain as greenhouse and open top chamber (OTC) study reports are ambiguous. Studies have yielded misleading results in the past regarding the effect of elevated [CO₂] on crop productivity, particularly in cases where pots restricted sink growth. To resolve these conflicting results, we compare the response of cassava to growth at ambient (ca. 385 ppm) and elevated [CO₂] (585 ppm) under field conditions and fully open air [CO₂] elevation. After three and half months of growth at elevated [CO₂], above ground biomass was 30% greater and cassava root tuber dry mass increased over 100% (fresh weight increased 89%). High photosynthetic rates and photosynthetic stimulation by elevated [CO₂], larger canopies, and a large sink capacity all contributed to cassava's growth and yield stimulation. Cassava exhibited photosynthetic acclimation via decreased Rubisco capacity early in the season prior to root tuber initiation when sink capacity was smaller. Importantly, and in contrast to a greenhouse study, we found no evidence of increased leaf N or total cyanide concentration in elevated [CO₂]. All of our results are consistent with theoretical expectations; however, the magnitude of the yield increase reported here surpasses all other C₃ crops and thus exceeds expectations.

Keywords: Africa, food security, harvest index, photosynthetic acclimation, sink strength, tropical crops

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Introduction

More than 900 million people are undernourished and nearly 90% live in Sub-Saharan Africa, Asia, and the Pacific (F.A.O., 2010). Sub-Saharan Africa has the greatest share of chronically hungry where approximately 240 million people are undernourished in terms of both food quantity and quality (F.A.O., 2010). However, precise estimates of future climate change impacts on crops in Africa are lacking due to a paucity of data on climate impacts on African crops (Lobell & Burke, 2010; Lobell *et al.*, 2008; Schlenker & Lobell, 2010). Given the

projections that future food production will need to double to meet the global demand by 2050 (Godfray *et al.*, 2010; Lobell *et al.*, 2008; Tilman *et al.*, 2011), there is an urgent need to assess the impact of climate change drivers on crops of food insecure regions.

The single most important driver of climate change is the globally increasing atmospheric [CO₂] (IPCC, 2007). Increasing atmospheric [CO₂] is predicted to stimulate photosynthesis, enhance water use efficiency, and increase yields in C₃ crops, such as rice, wheat, and soybean. A reassessment of empirical data has shown that the stimulation of C₃ crop productivity (i.e. rice, wheat, and soybean) by elevated [CO₂] may lead to much lower than predicted future yield stimulation (Ainsworth *et al.*, 2008; Long *et al.*, 2006). Ultimately,

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recent simulation models agree that atmospheric [CO₂] of ca. 550 ppm (a [CO₂] level expected by mid-century) will stimulate common C₃ crop (i.e. rice, wheat, and soybeans) productivity by 15–20% (Easterling *et al.*, 2007), which is considerably lower than theoretically predicted. The lower than predicted stimulation of crop yields by increasing [CO₂] is of particular concern for food security as most local and global model projections also agree that climate change will affect crop yields most severely in regions where undernourishment is already problematic (Easterling *et al.*, 2007; Godfray *et al.*, 2010; Lobell *et al.*, 2008).

Many undernourished populations rely on crops other than rice, wheat, or maize for their subsistence. In Sub-Saharan Africa, the starchy root tuber crop cassava accounts for almost two-thirds of the direct human caloric intake, whereas rice, wheat, and maize account for only one-third of total calories consumed (F.A.O., 2011). Cassava also plays an important role in food security and as a famine reserve crop. In areas where maize is preferred, but drought is recurrent, cassava is harvested when other crops fail (F.A.O., 2005), and cassava provides food security during armed conflicts when above ground crops are destroyed as the cassava tuber remains viable below ground for up to three years (Cock, 1982; Lebot, 2009). Even as it continues to be a vital subsistence crop for small scale farmers in Africa, global cassava production has also grown faster when compared to other root crops over the last decade, evidence that it is increasingly important at both regional and global scales (Fig. S1).

Cassava's importance as a food security crop is paradoxically hindered by inherent nutritional limitations as it has the lowest carbohydrate to protein ratio of the 10 most cultivated crops (Sayre *et al.*, 2011). A meal based solely on cassava tubers grown at today's [CO₂] only provides ca. 30% of the minimum daily protein requirement and substantially less of the daily zinc, iron, and vitamin A requirements (Sayre *et al.*, 2011). In addition, cassava produces a cyanogenic glycoside called linamarin along with lesser amounts of its methyl homologue lotaustralin, and root tubers can have cyanide concentrations as high as 500 mg/Kg in bitter varieties or be lower than 100 mg/Kg in sweet varieties (McMahon *et al.*, 1995). Diets high in sulfur containing proteins facilitate the detoxification of cyanogenic glycosides, but growth at elevated [CO₂] reduces plant protein content (Taub *et al.*, 2008); consequently, small cyanide doses in improperly or incompletely processed cassava can accumulate and lead to cyanide toxicity in protein deficient populations (Cliff *et al.*, 1985; Mckey *et al.*, 2010).

Growth at elevated [CO₂] significantly alters leaf chemistry (Gleadow *et al.*, 1998), but the net effect that

growth at future elevated atmospheric [CO₂] will have on plant cyanogenic glycosides is still not well understood. Cyanogenic glycosides in white clover (*Trifolium repens*) did not increase at an elevated [CO₂] of 600 ppm when grown at ETH-FACE (Frehner *et al.*, 1997) or when grown in pots in a greenhouse at 700 ppm [CO₂] (Gleadow *et al.*, 2009a). However, a recent greenhouse study on cassava reported that decreased yields at elevated [CO₂] were accompanied by increases in leaf, but not tuber, cyanide content (Gleadow *et al.*, 2009b). The results of the latter study are puzzling because they are inconsistent with the notion that elevated [CO₂] will stimulate C₃ crop productivity (Leakey *et al.*, 2009; Long *et al.*, 2006) and alarming because cassava leaves are eaten for their protein, and a higher leaf cyanide content suggests increased toxicity at elevated [CO₂]. Whether increases in biomass or leaf toxicity would occur in cassava under field conditions and fully open air [CO₂] elevation remains unknown.

Cassava's potential to enhance food security under climate change may well be underestimated (Rosenthal & Ort, 2012). Due to their ability to store carbohydrates throughout most of the growing season, tuberous storage organs as found in cassava are inherently strong photosynthate sinks. Photosynthetic regulation depends in part on the balance between the substrate for photosynthesis (i.e. CO₂) and the sink capacity for photosynthates (reviewed in Paul & Foyer, 2001). Thus, all else being equal, plants with large vegetative sinks that are unrestricted and available throughout the growing season should exhibit greater stimulation in response to elevated [CO₂] than plants with shorter lived reproductive sinks (Arp, 1991; De Temmerman *et al.*, 2007; Miglietta *et al.*, 1998). Tubers also have the potential for increases in the ratio of edible to nonedible components (i.e. harvest index) under elevated [CO₂], whereas the harvest index for above ground crops frequently decreases under elevated [CO₂] (Ainsworth & Long, 2005; Kimball *et al.*, 2002).

In spite of cassava's importance in food insecure regions and its potential beneficial attributes in the context of climate change, remarkably little is known about cassava's response to atmospheric global change factors under field conditions. Here, we begin addressing these knowledge gaps by assessing the response of cassava to growth under fully open air [CO₂] elevation (FACE). As there are no FACE facilities in a tropical agricultural setting where cassava is cultivated, the experiment was conducted at SoyFACE, in Illinois, USA. We tested the response of cassava cultivar 40333, which is the subject of global biofortification efforts aimed at enhancing nutrition and decreasing cyanogenic glycosides in cassava (Abhary *et al.*, 2011; Narayanan *et al.*, 2011; Sayre

et al., 2011). The objectives of this study were to: 1) test the hypothesis that cassava's substantial sink capacity would enhance yields more than other C_3 crops grown under FACE, 2) assess whether photosynthetic acclimation would reduce cassava's stimulation by elevated $[CO_2]$, and 3) evaluate whether cyanide content would increase for cassava plants grown in an unrestricted soil volume under fully open air $[CO_2]$ elevation.

Materials and methods

Site description and plant materials

Cassava was grown within subplots of eight 300 m² corn plots (rings), set within a typically managed corn field of ca. 20 hectares (ha) at the SoyFACE facility located in the Experimental Research Station of the University of Illinois at Urbana-Champaign (Leakey *et al.*, 2004). The air of four "elevated" plots was enriched with pure CO_2 , and four "ambient" plots served as the nonelevated controls (Morgan *et al.*, 2005). In the elevated $[CO_2]$ plots, plants were enveloped by air at a $[CO_2]$ of 200 ppm above ambient. Ambient atmospheric $[CO_2]$ at the beginning of the 2010 field season was ca. 385 ppm; therefore the target $[CO_2]$ for elevated plots in 2010 was 585 ppm $[CO_2]$. Elevated plots were enriched using a modification in the method of Miglietta *et al.* (2001) and described in detail by Ort *et al.* (2006). In the elevated plots, 75% of $[CO_2]$ values recorded every 10 min across the growing season in 2010 were within 10% of the target value. The mean daily $[CO_2]$ in elevated plots was 578.9 ± 16 (SD). The plots were not enriched with CO_2 at night.

On May 4, 2010, cassava stems from a single clone (*Manihot esculenta*, cv. 60444) were planted in potting media (Sunshine Mix; Sungro Horticulture, Bellevue Washington) and allowed to sprout in the greenhouse. Three weeks later plants were moved outside to harden for 1 week. On June 4, 2010, 30 days after planting (DAP), plants had 3–5 true leaves and were transplanted into the field. Nine cassava plants were transplanted to a nine square meter subplot within each of the eight larger corn plots. Cassava plots were monitored regularly for weeds and herbivore damage. Weeds were removed manually as they emerged, and vials containing predator urine were placed in each plot to deter small herbivores. In early May of 2010, prior to planting corn, all plots were fertilized at ca. 150 kg of N per hectare.

Gas exchange measurements and carbon gain estimates

Gas exchange was measured on fully expanded, nonsenescent "sun" leaves at the top of the canopy. The diurnal course of cassava photosynthesis (A) was measured at growth $[CO_2]$ at five time points during six days in midsummer 2010 (June 29, July 6, July 21, August 5, August 19, and August 31). To ensure that each plant was measured under similar environmental conditions during each time point, the LED illumination system of the controlled environment cuvettes of the gas exchange system (LI-6400, LI-COR, Lincoln, Nebraska) were

set to deliver the same ambient light levels; in addition, temperature and relative humidity were also set to ambient conditions and kept constant for the duration of each time point in the diurnal. Photosynthetic measurements were made on three plants in each plot at each time point. Plots were randomly sampled within each time point to avoid confounding treatment with time effects within a sampling. Each measurement took approximately 2 min, so each time point lasted about 50 min. Statistical analyses were performed on plot means for four ambient $[CO_2]$ plots and four elevated $[CO_2]$ plots.

To estimate the total daily carbon gain of sun leaves at the top of the canopy, the daily integral of photosynthesis (A') was calculated as the area below the diurnal data in Fig. 2. Photosynthesis was assumed to increase linearly from 0 $\mu\text{mol } CO_2 \text{ m}^{-2} \text{ s}^{-1}$ at dawn (sunrise) to the first measured value and decrease linearly from the last measured values to 0 $\mu\text{mol } CO_2 \text{ m}^{-2} \text{ s}^{-1}$ at sunset. Extrapolation of A in the early morning was necessary as dew on the leaves prevented measurement of photosynthesis. We then estimated A' for each plot by integration using the trapezoidal rule and performed statistical analyses on the integrals as described below.

Photosynthetic response to the internal $[CO_2]$ of the leaf (i.e. A vs. C_i curves) was measured with an open gas exchange system (LI-6400, LI-COR, Lincoln, Nebraska) in the field on five dates (July 5, July 19, August 7, August 15, August 31) just before or just after the diurnal series measurements. Leaves were allowed to reach steady state photosynthesis at their growth $[CO_2]$ and at a saturating light level of 1500 $\mu\text{mol } \text{m}^{-2} \text{ s}^{-1}$. Once steady state was attained, photosynthetic CO_2 uptake rate (A) and chlorophyll fluorescence parameters were recorded, and thereafter the reference $[CO_2]$ was decreased in steps to 50 ppm, returned to growth $[CO_2]$, and then increased stepwise to 2000 ppm. A minimum of 12 data points were collected for each plant following the methods outlined by Long & Bernacchi (2003). Over all of the A vs. C_i determinations, mean leaf-to-air vapor pressure deficit (VpdL) was 1.61 ± 0.14 kPa (SD), and mean leaf temperature was 29.9 ± 1.16 °C (SD). Maximum carboxylation capacity ($V_{c,max}$), maximum potential linear electron flux (J_{max}), and respiration in the light (R_d) were calculated by fitting individual A vs. C_i curves to a biochemical model of photosynthesis (Farquhar *et al.*, 1980) using the method described in Long & Bernacchi (2003). As a result of day to day variation in leaf temperature, all parameters are reported as their value at 25 °C (i.e. $V_{c,max@25}$, $J_{max@25}$) (Bernacchi *et al.*, 2001, 2003).

Growth determination and tissue sampling

Plant growth and phenology were monitored and measured every 7–14 days. Plant height was measured from the ground to the uppermost growing point. Number of nodes and stem diameter of all individuals were also measured every 7–14 days. The length, width, and height of each plant's canopy were used to calculate the volume of an ellipsoid [$4/3 \pi$ (Plant Height/2) (Canopy Length/2) (Canopy Width/2)], which was taken as an estimate of plant size above the ground.

Leaf disks collected after 67 and 103 days of [CO₂] elevation (DOE) (August 9 and September 15) were dried at 60 °C and weighed to determine specific leaf area (SLA, m² kg⁻¹). These same disks were then ground to a fine powder and used to determine leaf carbon (C) and nitrogen (N) content. Two additional leaf disks were collected and analyzed for leaf cyanide content following Gleadow *et al.* (2010). Briefly, these disks were placed directly into 0.1 M citrate buffer (pH 5.5) in a 2 ml microcentrifuge tube above which a small well containing 1 M NaOH was suspended. The cyanide evolved from the leaf tissue was trapped in the 1 M NaOH well and was quantified using a colorimetric assay modified for a microplate reader. Prior to quantification, the samples were subjected to two freeze-thaw cycles to lyse the cells and facilitate mixing of the cyanogenic glycosides and endogenous leaf degradative enzymes. Samples were then incubated for 20 h at 37 °C, and their absorbances were read at 595 nm using a KCN standard. Leaf disks were rinsed to remove the citrate buffer, oven dried, and weighed. Leaf cyanide content is reported in mg of cyanide per gram of dry weight.

Biomass and yield

The root tuber fresh weight and total leaf area were determined immediately following the harvest. Stems, leaves, and root tubers were separated and dried at 70 °C for 72 h prior to determining dry mass. Final leaf area index (LAI) was estimated for each plot as the ratio of the total leaf area per plot divided by the total plot area.

Statistical analysis

Leaf traits (carbon, nitrogen, cyanide content, and final harvest data) were analyzed using a mixed model analysis of variance (PROC MIXED; SAS) with [CO₂] (ambient or elevated) as a fixed effect and block ($n = 4$) as a random effect. The diurnal course of photosynthesis at SoyFACE was analyzed using a repeated measures mixed model analysis of variance (PROC MIXED; SAS) with [CO₂] (ambient or elevated) as a fixed effect, time of day (Tpoint) as the repeated factor, and block as a random factor. Plant growth data, daily integrated photosynthesis (A'), and photosynthetic parameters ($V_{c,max@25}$, $J_{max@25}$) were analyzed with repeated measures mixed model analysis of variance as above, but with the day of year (DOY) as the repeated factor. As there were only four blocks, significant probability was set at $P < 0.1$ a priori to reduce the likelihood of type II errors (Scheiner, 2001; Sokal & Rohlf, 1995).

Results

Site climate

The average annual precipitation in Champaign-Urbana is 1041 mm and the average growing season precipitation from June to September is 377 mm (Illinois State Water Survey, 2011). From June 1 to September

30, 2010, 428 mm of precipitation was recorded (Fig. S2). During the experiment (June 4 to September 15) the mean daily minimum temperature was 18.23 ± 3.3 (SD) °C (Illinois State Water Survey). The mean daily maximum temperature of 29.9 ± 2.8 (SD) °C was optimal for cassava growth (i.e. >25 °C) on 96% of days. Temperatures below 15 °C inhibit cassava growth (Alves, 2002). The air temperature dipped below 15 °C for some period of time on 22 separate days with half of these occurring in sequence just prior to harvest. The daily mean incident solar radiation at SoyFACE from June 4 to September 15, 2010 of $26.35 \text{ MJ m}^{-2} \text{ day}^{-1}$ was comparable to that available between 0° and 15° latitude where most cassava is grown.

Growth and phenology

The first six weeks in the field, growth was similar in ambient and elevated [CO₂] treatments (Fig. 1). After eight weeks in the field, cassava growth was significantly greater in elevated [CO₂] plots (Table 1, Fig. 1). Height and node number in elevated [CO₂] was significantly greater than for ambient plants after July 27 (DOY 208) (Table 1, Fig. 1). The size of each plant's canopy, estimated as the volume occupied by each plant, was significantly greater in elevated [CO₂] from August 4 (DOY 216) onward (Table 1, Fig. 1).

Diurnal course of gas exchange

Elevating [CO₂] significantly increased instantaneous photosynthetic rate (A) of upper canopy leaves on all dates except on June 29 when the stimulation by [CO₂] was only marginally significant (Table 2, Fig. 2). Photosynthetic stimulation was greatest just before or immediately following midday. The daily photosynthetic integral (A') was significantly greater in elevated [CO₂] on all dates ($F_{1,43.1} = 77.99$; $P < 0.0001$). A significant date by [CO₂] interaction ($F_{5,43.1} = 2.52$; $P < 0.0001$) revealed that the degree of stimulation of A' varied by date (Fig. 2 see insets). Averaged across all measurement dates, A' was $29\% \pm 5.1\%$ greater in elevated [CO₂] with the greatest increase in A' on July 5 (52.6%) and the lowest on August 5 (14.6%). During the first three diurnal measurement series, there were no detectable differences in stomatal conductance (g_s) between plants grown in ambient and elevated [CO₂], but g_s was significantly lower in elevated [CO₂] plots on the last three diurnal series (Table 2, Fig. 3). Instantaneous water use efficiency, the ratio of carbon gained (A) to calculated water lost via transpiration (i.e. g_s), was significantly higher in elevated [CO₂] on all six dates, and this effect was consistent throughout the day (Table 2, Fig. 4).

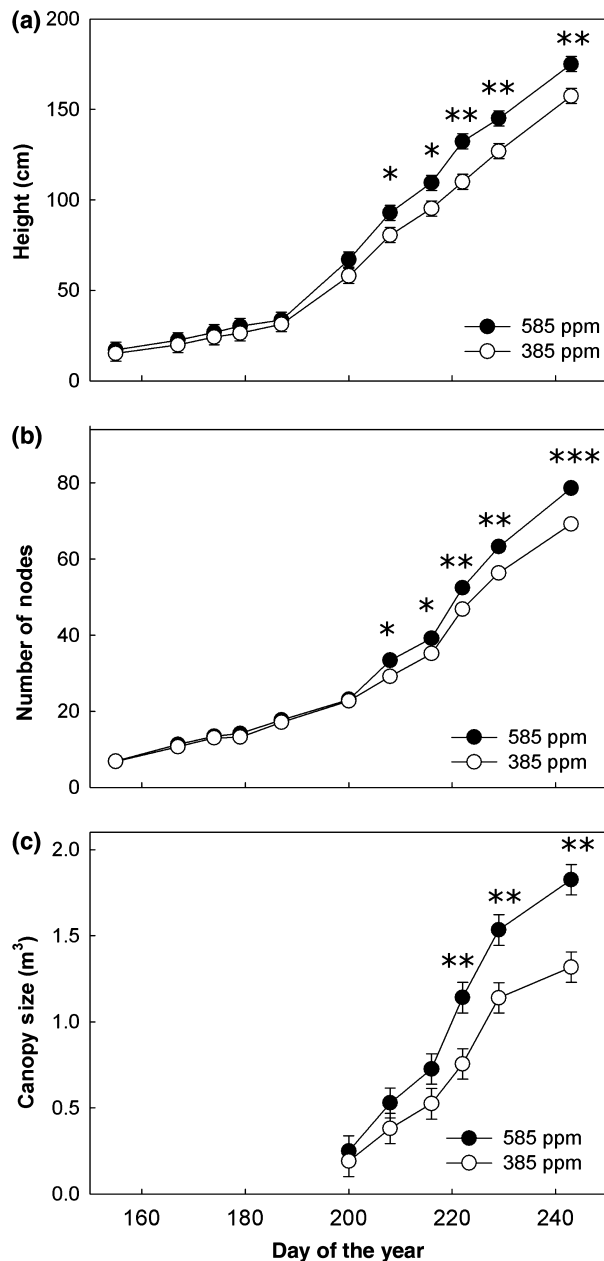


Fig. 1 Height (a), number of nodes (b), and canopy size (c) were measured from just after planting through to two weeks before harvest. Canopy volume was calculated as the volume of an ellipsoid (see Methods). Means are for $n = 4$ blocks (\pm SE) in ambient (open circles) and elevated $[\text{CO}_2]$ (closed circles). In some cases, error bars are smaller than the symbols. Asterisks denote significant differences between treatments on a given date (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Photosynthetic parameters

Photosynthetic parameters derived from A vs. Ci curves were measured five times during the experiment and were all within two days of diurnal series

measurements. Across the growing season elevating $[\text{CO}_2]$ decreased maximum carboxylation capacity ($V_{c,\text{max}@25}$) by 6.5%. The response of $V_{c,\text{max}@25}$ to $[\text{CO}_2]$ differed over time (Table 3, Fig. 5a). $V_{c,\text{max}@25}$ was significantly lower on the first three measurement dates, July 5, 19, and August 7 but not on the last two where there was no difference. In contrast, there were no detectable differences in maximum linear electron flux through photosystem II ($J_{\text{max}@25}$) between ambient and elevated plants on the first three dates, but $J_{\text{max}@25}$ was significantly greater in elevated $[\text{CO}_2]$ plots on the last two measurement dates (Table 3, Fig. 5c). Elevating $[\text{CO}_2]$ decreased the average ratio of $V_{c,\text{max}@25}/J_{\text{max}@25}$ (V/J) between 5.5% and 18% (Table 3, Fig. 5e); consequently, the transition from Rubisco to RuBP limited photosynthesis (C_i at inflection), estimated from $V_{c,\text{max}@25}$, $J_{\text{max}@25}$, and R_d derived from individual A vs. C_i curves, was consistently higher for plants grown in elevated $[\text{CO}_2]$ (Table 3, Fig. 5g and right panels).

Leaf traits

The area per unit mass or specific leaf area (SLA) was significantly lower for plants grown at elevated $[\text{CO}_2]$ on both measurement dates (Fig. 6A). Leaf nitrogen content (%N) on a dry mass basis was significantly lower leading to a significant increase in the ratio of carbon to nitrogen (C : N) on both dates (Table 4, Fig. 6B and C). There was no detectable effect of $[\text{CO}_2]$ on leaf cyanide content measured on the two dates, but cyanide content was greater in September than in August (Table 4, Fig. 6D).

Biomass allocation and final yield

Total above ground biomass after three and half months growth in the field was 30% greater for plants grown at elevated $[\text{CO}_2]$ (Fig. 7A). Leaf biomass per plant was 20% greater ($F_{1,3} = 14.19$; $P < 0.05$), and stem biomass was 34% greater in elevated when compared with ambient $[\text{CO}_2]$ ($F_{1,3} = 6.83$; $P < 0.06$). Average dry mass of the root tubers increased 104% from 119.6 g in ambient plots to 244 g per plant in elevated $[\text{CO}_2]$ plots ($F_{1,3} = 35.75$; $P < 0.01$). The root tuber fresh mass yield (tons ha^{-1}) was 89% greater in elevated $[\text{CO}_2]$ plots ($F_{1,3} = 46.3$; $P < 0.01$) (Fig. 7C). The average leaf area index (LAI) at harvest was 3.00 ± 0.12 for ambient plots and 3.53 ± 0.12 for elevated plots ($F_{1,3} = 35.24$; $P < 0.01$) (Fig. 7B). The resulting increase in tuber yield led to a 50% increase in the biomass allocated to tubers (i.e. tuber dry weight/total dry weight) and a 57.5% increase in the harvest index (i.e. tuber dry weight/

Table 1 Repeated measures ANOVA of the effects of day of year (DOY) and [CO₂] on growth of cassava plants at ambient and elevated [CO₂] at SoyFACE. Degrees of freedom (df) vary because growth and node number were measured on 11 dates, whereas canopy volume was estimated on six dates. Canopy size was estimated as the volume of an ellipsoid (see Methods for details)

	Height			Node number			Canopy size		
	df	F	P	F	P	df	F	P	
[CO ₂]	1,8	4.77	0.0604	6.39	0.0354	1,8	6.19	0.0377	
DOY	10,80	670.71	0.0001	1366.37	0.0001	5,40	344.25	<.0001	
DOY*[CO ₂]	10,80	3.41	0.0009	7.29	0.0001	5,40	9.28	<.0001	

Bold values are significant at $P < 0.01$.

Table 2 Repeated measures ANOVA of the effects of [CO₂] and time of day (Tpoint) on diurnal variation in photosynthesis (A), stomatal conductance (g_s), and instantaneous water use efficiency (A/g_s) on four dates in 2010. Photosynthesis was measured five times (Tpoint) on each day except for July 21 when inclement weather halted sampling after the third time point

	A			g _s			A/g _s		
	df	F	P	df	F	P	df	F	P
June 29									
[CO ₂]	1,8	4.44	0.0682	1,4	0.11	0.7553	1,36	25.29	0.0001
Tpoint	4,32	7.9	0.0002	4,32	2.59	0.0552	4,36	0.53	0.7133
Tpoint*[CO ₂]	4,32	0.13	0.9719	4,32	0.69	0.6051	4,36	1.28	0.2948
July 6									
[CO ₂]	1,4	21.94	0.0094	1,8	0.2	0.6689	1,8.01	8.92	0.0174
Tpoint	4,32	13.76	<0.0001	4,32	10.11	<0.0001	4,32	6.2	0.0008
Tpoint*[CO ₂]	4,32	0.53	0.7142	4,32	0.72	0.5817	4,32	1.95	0.1253
July 21									
[CO ₂]	1,8.4	47.71	<.0001	1,4.06	0.21	0.6688	1,7.34	28.45	0.0009
Tpoint	2,16.3	8.67	0.0027	2,15.7	0.08	0.9205	2,14.9	8.97	0.0028
Tpoint*[CO ₂]	2,16.3	0.65	0.533	2,15.7	0.6	0.5626	2,14.9	1.22	0.3236
August 5									
[CO ₂]	1,8	14.13	0.0055	1,8	21.68	0.0016	1,8.02	19.67	0.0022
Tpoint	4,32	30.04	0.0001	4,32	74.92	0.0001	4,32	16.21	0.0001
Tpoint*[CO ₂]	4,32	3.86	0.0114	4,32	3.79	0.0125	4,32	7.84	0.0002
August 19									
[CO ₂]	1,4.4	27.34	<.0001	1,4	7.31	0.0539	1,8.1	61.77	<.0001
Tpoint	5,4.4	43.41	<.0001	5,4.0	21.45	<.0001	5,39.9	17.39	<.0001
Tpoint*[CO ₂]	5,4.4	1.87	0.1199	5,4.0	0.54	0.7412	5,39.9	3.05	0.0202
August 31									
[CO ₂]	1,4	67.55	0.0012	1,4	52.28	0.0019	1,4	282.57	<.0001
Tpoint	4,32	21.13	<.0001	4,32	3.77	0.0127	4,32	0.6	0.6623
Tpoint*[CO ₂]	4,32	1.17	0.3437	4,32	0.48	0.7529	4,32	0.06	0.9932

Bold values are significant at $P < 0.01$.

above ground dry biomass) (Fig. 7D, $F_{1,3} = 17.39$; $P < 0.05$).

Discussion

The objectives of this study were 1) to determine whether photosynthesis, daily carbon gain, and yield would increase more for cassava than other C₃ crop plants grown under fully open air [CO₂] elevation, 2) to assess if photosynthetic acclimation to elevated [CO₂]

would diminish the stimulatory effect of [CO₂] in the field, and 3) to evaluate whether leaf and root tuber cyanide content would increase in cassava plants grown at elevated [CO₂].

FACE elevated [CO₂] stimulated cassava yield more than for any other C₃ crop

The expected physiological response to elevated [CO₂] in cassava was coupled with an uncommon combination

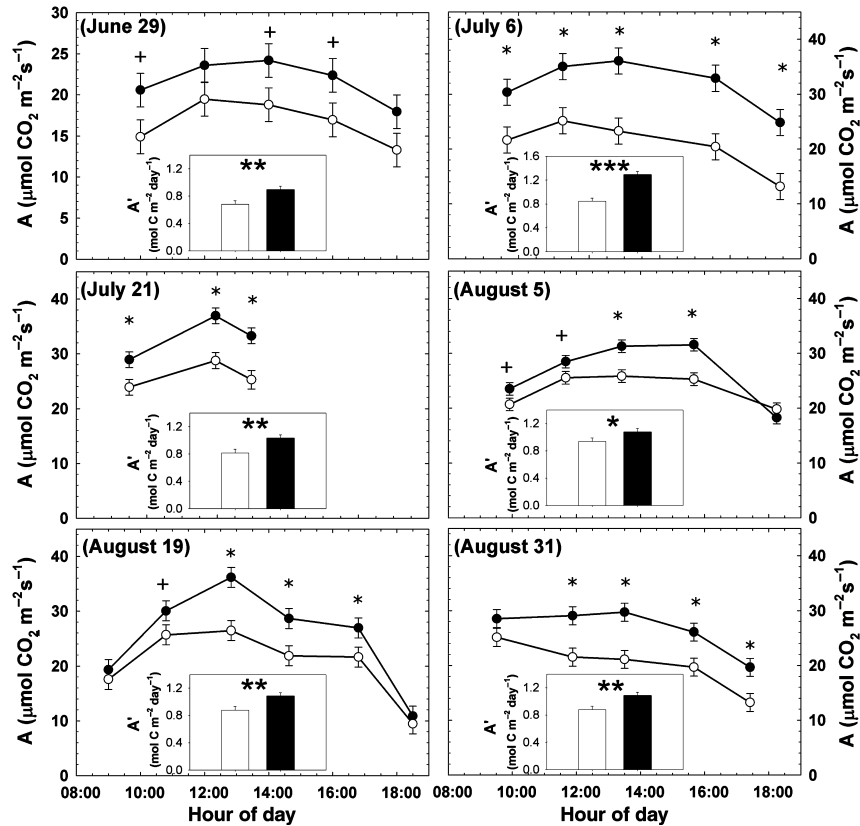


Fig. 2 Diurnal course of photosynthesis of cassava plants grown at SoyFACE in ambient (385 ppm, open circles) and elevated (585 ppm, closed circles) $[\text{CO}_2]$ on six dates in 2010. Insets: daily photosynthesis integral (A') for ambient $[\text{CO}_2]$ (white bars) and elevated $[\text{CO}_2]$ (black bars). Symbols are means for $n = 4$ block (\pm SE). Asterisks (see figure 1 for P values) and crosses ($P < 0.1$) denote significant differences between treatments on a given date and time. Note: scale for A differs on June 29.

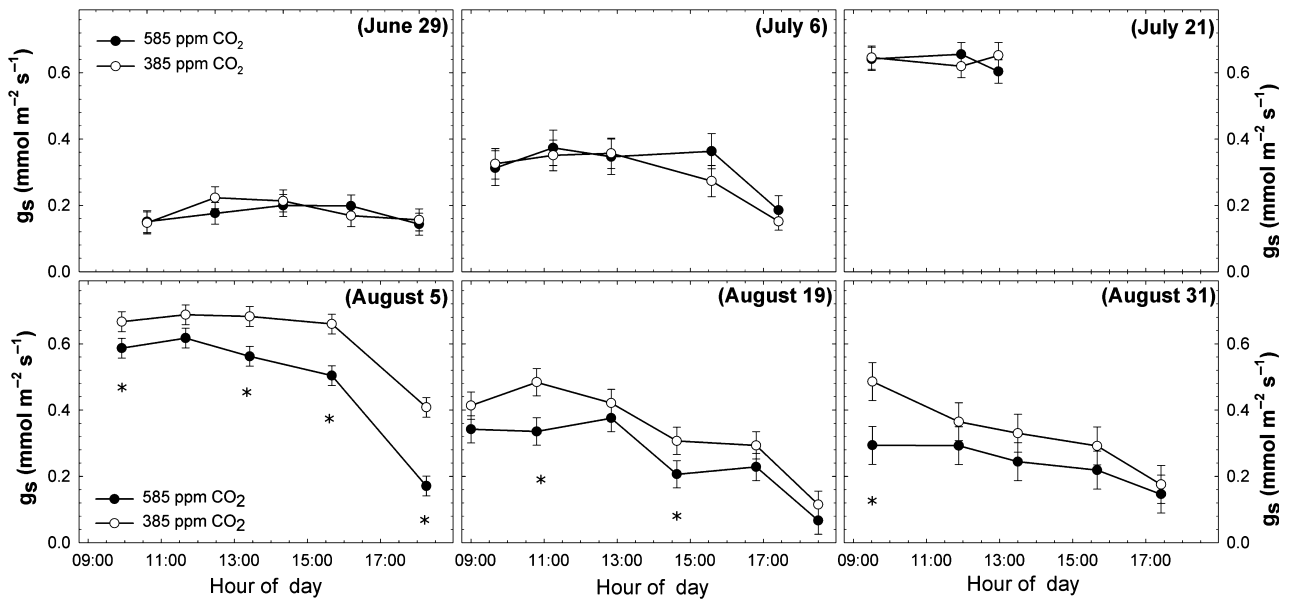


Fig. 3 Diurnal course of stomatal conductance (g_s) for cassava plants grown at SoyFACE in ambient (385 ppm, open circles) and elevated (585 ppm, closed circles) $[\text{CO}_2]$ on six dates in 2010. Symbols are means for $n = 4$ blocks (\pm SE). Asterisks (see figure 1 for P values) denote significant differences between treatments on a given date and time.

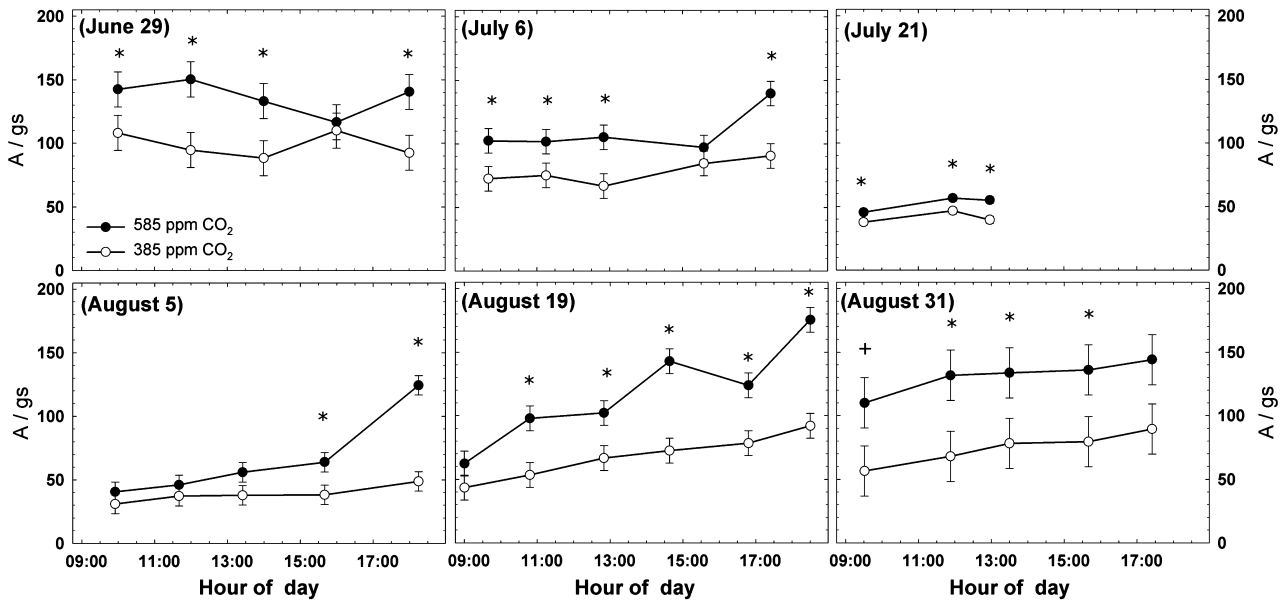


Fig. 4 Diurnal course of instantaneous water use efficiency (A/g_s) of cassava plants grown at ambient (385 ppm, open circles) and elevated (585 ppm, closed circles) $[CO_2]$ on six days in 2010. Symbols are means for $n = 4$ blocks (\pm SE). Plants grown and measured at elevated $[CO_2]$ had significantly lower A/g_s on all dates. Asterisks (see figure 1 for P values) and crosses ($P < 0.01$) denote significant differences between treatments on a given date and time.

Table 3 ANOVA of the effects of $[CO_2]$ and day of year (DOY) on biochemical parameters of photosynthesis estimated from A vs. C_i response curves. Maximum carboxylation capacity of Rubisco ($V_{c,max@25}$), maximum linear electron flux through photosystem II ($J_{max@25}$), the ratio of $V_{c,max@25}/J_{max@25}$ (V/J), and the point at which photosynthesis shifts from Rubisco limitation to RuBP limitation (C_i at inflection)

	$V_{c,max@25}$			$J_{max@25}$			V/J			C_i at inflection		
	df	F	p	df	F	p	df	F	p	df	F	p
$[CO_2]$	1,7.93	7.45	0.0261	1,8.27	3	0.12	1,8.3	20.1	0.0019	1,4.06	57.8	0.0015
DOY	4,32.3	10.05	<.0001	4,31.6	2.89	0.0378	4,31.7	8.99	<.0001	4,32	4.88	0.0035
DOY* $[CO_2]$	4,32.3	4.16	0.0079	4,31.6	2.14	0.0986	4,31.7	2.14	0.0987	4,31.9	1.89	0.1359

Bold values are significant at $P < 0.01$.

of traits that interacted to cause an unexpectedly large yield stimulation under field conditions. Notably, several of cassava's physiological responses to growth at elevated $[CO_2]$ were comparable to other C_3 crops. For instance, midday photosynthetic rates (A) at saturating light and growth $[CO_2]$ in the field were 20–54% greater (mean = 35% \pm 4.7%) in elevated $[CO_2]$ plots (Fig. 2), an increase in A which is similar to potato (Vaccari *et al.*, 2001) and other C_3 crops in FACE studies grown with ample nitrogen and without drought stress (Ainsworth & Long, 2005; Ainsworth & Rogers, 2007; Kimball *et al.*, 2002; Rosenthal *et al.*, 2011). Similarly, the daily integral of photosynthesis of the canopy sun leaves (A') was 29.6% \pm 5.1% higher in elevated $[CO_2]$ when compared with ambient $[CO_2]$ plots and is also comparable to the average stimulation for C_3 crops in

other FACE experiments (Ainsworth & Long, 2005). Cassava's light saturated photosynthetic capacity, although not inherently greater than many C_3 crops, is almost twice that of potato at ambient $[CO_2]$ (e.g. Lawson *et al.*, 2001; Vaccari *et al.*, 2001). Thus, while cassava's proportional increase in photosynthesis is similar to other crops, its high photosynthetic rate is one trait that contributed to greater total stimulation by elevated $[CO_2]$ when compared with potato.

The increase in photosynthesis and biomass in cassava at elevated $[CO_2]$ implies an increased efficiency with which intercepted solar radiation was converted into biomass (i.e. ϵ_c) (Monteith, 1972, 1977), which may also increase for all C_3 plants grown in FACE (Dermody *et al.*, 2008). An estimate of ϵ_c for cassava can be made using the energy content of total harvested cassava

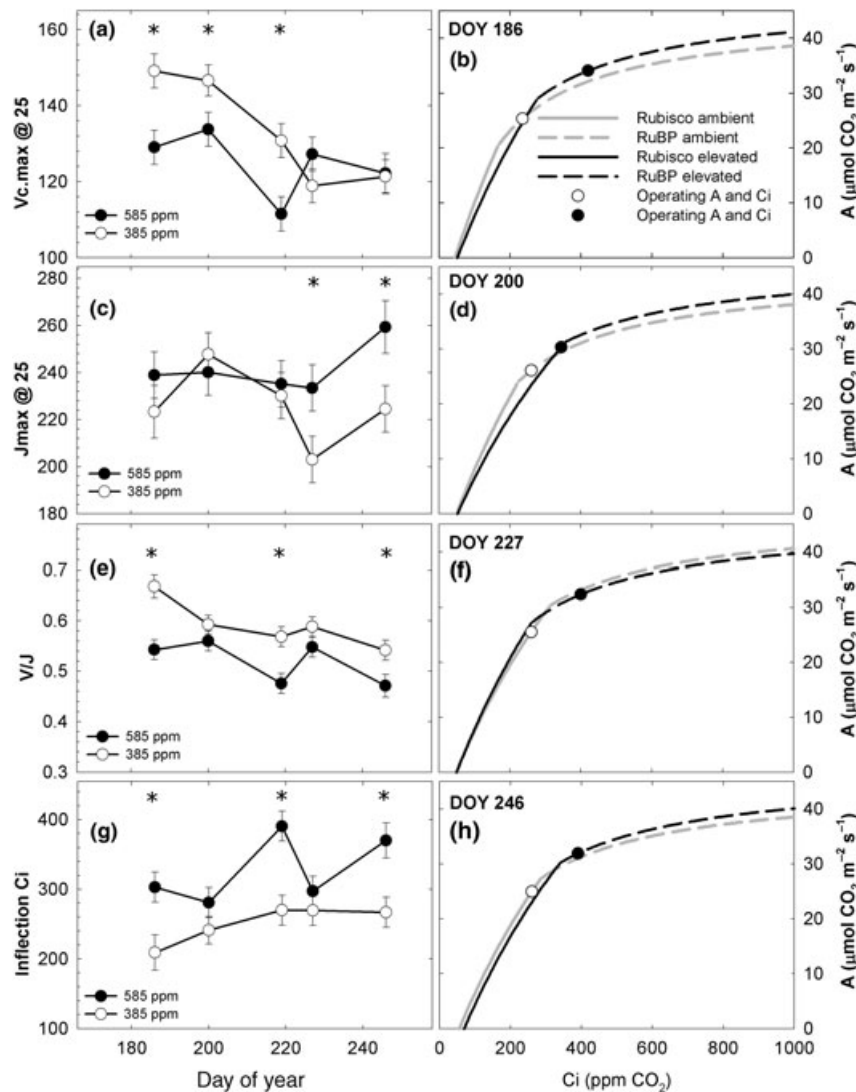


Fig. 5 Photosynthetic parameters (a) $V_{c,max@25}$, (c) $J_{max@25}$, (e) $V_{c,max@25}/J_{max@25}$, (g) C_i of transition between Rubisco and RuBP limited photosynthesis (A) of cassava plants grown in ambient (385 ppm, open circles) and elevated (585 ppm, closed circles) $[CO_2]$ measured on five dates in 2010. Symbols are means for $n = 4$ plots (\pm SE). Asterisks (see figure 1 for P values) denote significant differences between treatments. Right panels: modeled photosynthesis vs. C_i curves on four dates (b) July 5, (d) August 7, (f) August 15, and (h) September 3. Curves were generated using the model of Farquhar *et al.* (1980) and parameters from the left panels on the corresponding day of year (see Methods for details). The symbols on the curves represent the operating photosynthesis and C_i under field conditions at saturating light levels. All values and parameters were normalized to 25 °C using Bernacchi *et al.* (2001, 2003).

biomass (calculated at 16.8 MJ Kg⁻¹) (Connor *et al.*, 1981). The mean daily solar incident radiation for the 103 days in the field was 26.35 MJ m⁻² day⁻¹; thus, we estimate that ϵ_c increased from 0.37% of the total incident radiation at ambient $[CO_2]$ to 0.54% in elevated $[CO_2]$ plots. Published estimates of ϵ_c also based on incident solar radiation for cassava at ambient $[CO_2]$ range between 0.26% and 0.38% up to 100 days after planting and 0.64% and 1.1% from 150 to 220 days after planting, depending on the cultivar (Connor *et al.*, 1981). Lower estimates of ϵ_c for cassava plants at SoyFACE when compared with other studies at

ambient $[CO_2]$ could be due to cultivar differences, a truncated growing season (see below), or unmeasured litter loss.

Cassava had increasingly taller plants with larger canopies as the season progressed, leading to a significantly greater LAI in elevated $[CO_2]$ plots at harvest. Growth at elevated $[CO_2]$ has reportedly accelerated canopy senescence in other tuber crops (Manderscheid *et al.*, 2010; Miglietta *et al.*, 1998, 2000). There was no evidence of accelerated canopy senescence at SoyFACE or for cassava grown in open top chambers (OTC) at 685 ppm $[CO_2]$ (Fernandez *et al.*, 2002). As the growing

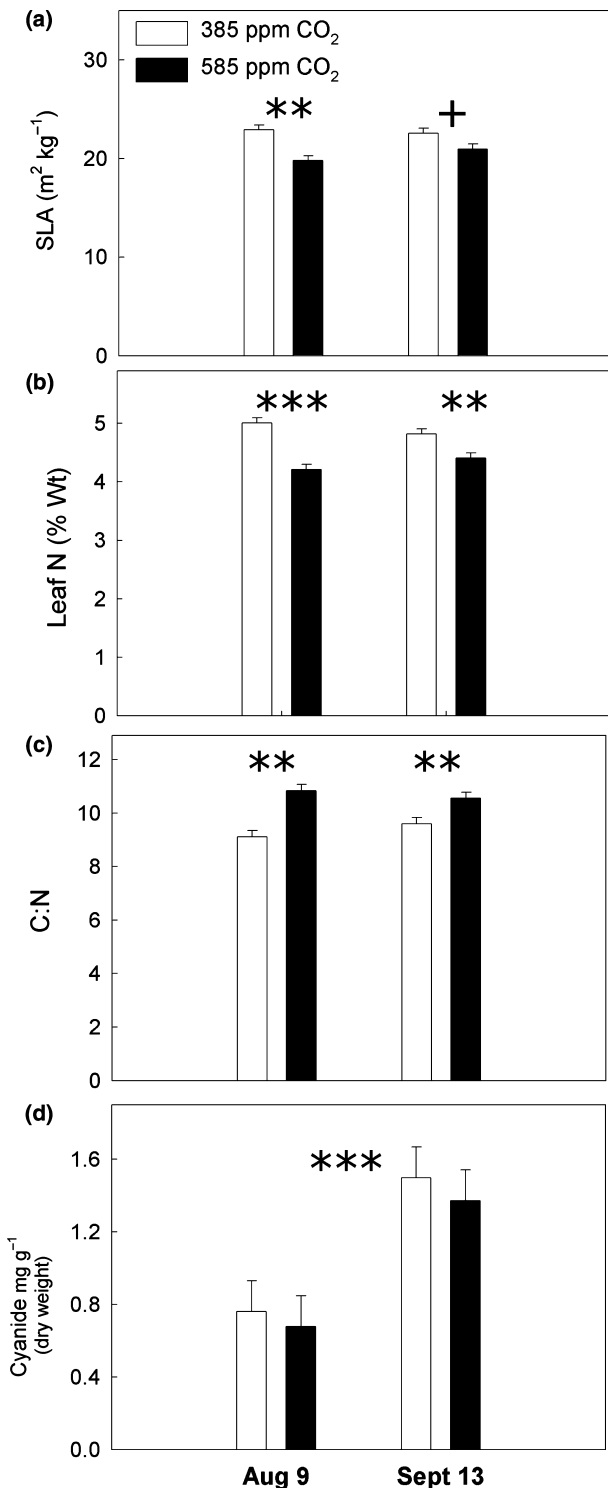


Fig. 6 Specific leaf area, nitrogen content, carbon nitrogen ratios, and cyanide content of mature fully expanded leaves of cassava plants grown at ambient (white bars) and elevated (black bars) [CO₂]. Bars are means for $n = 4$ plots. Panels (a) to (c) asterisks denote significant difference between ambient and elevated [CO₂] plots. Panel (d) asterisks denote differences between sampling dates (see figure 1 for P values).

season here was cut short we may not have been able to detect either [CO₂] induced or delayed senescence. Nevertheless, the cassava canopy was equally robust in ambient and elevated [CO₂] plots throughout the experiment. In contrast, the canopies of potato and sugar beet, the only other tuberous crops tested in FACE, both senesced earlier and LAI decreased faster prior to harvest at elevated [CO₂] (Manderscheid *et al.*, 2010; Miglietta *et al.*, 1998, 2000). In the cassava OTC experiment, Fernandez *et al.* (2002) reported significant increases in LAI, but only after 60 days of [CO₂] elevation (DOE) at an elevated [CO₂] of 680 ppm. Above ground growth, canopy size, and node number were all similarly unaffected by elevated [CO₂] for cassava at SoyFACE during the first two months in the field. In contrast, growth, node number, and canopy size were all significantly greater in elevated [CO₂] plots after 60 DOE. As each node adds a new leaf, and there were no visible signs of accelerated senescence in elevated [CO₂] plots, LAI was likely greater in elevated plots from midsummer until harvest. Although it is not always true (reviewed in Ainsworth & Long, 2005), greater LAI and leaf area duration both contributed to increases in biomass and yield for cassava at SoyFACE, which is consistent with other FACE experiments (Dermoddy *et al.*, 2006; Franzaring *et al.*, 2010; Morgan *et al.*, 2005).

After three and half months of growth, cassava above ground dry matter production had increased by 30% in elevated [CO₂] plots when compared with ambient controls, significantly more than the 17% average increase in C₃ plants grown at FACE sites, but similar to the 28% increase reported for trees (Ainsworth & Long, 2005). Final root tuber biomass (yield) increased by 89% (=105% in dry mass), more than four times that of potato and more than any crop grown in a FACE experiment. The next highest yield stimulation by elevated [CO₂] in FACE for a C₃ crop after cassava is 42%, reported for cotton (Mauney *et al.*, 1994). Yields of potato were only stimulated by 21% at an elevated [CO₂] comparable to that used in our cassava experiment (i.e., ~550 ppm); however, potato yields were stimulated 40% when grown at 660 ppm (Miglietta *et al.*, 1998). Sugar beet, the only other tuberous crop grown in a FACE experiment, had yields that were only stimulated 17% under high N conditions and elevated [CO₂] (Manderscheid *et al.*, 2010).

Why does cassava have a greater stimulation than any other tuber crop grown under fully open air [CO₂] fumigation? Cassava's advantage over potato and sugar beet is most likely due to greater photosynthetic rate, consistently larger canopy in elevated [CO₂], and probably a longer period of high photosynthetic capacity. Cassava also has unusually long lived leaves that

Table 4 ANOVA for the effects of [CO₂] and date on specific leaf area (SLA), leaf nitrogen content (Leaf N), carbon to nitrogen ratio (C:N), and leaf cyanide content

	df	SLA		Leaf N		C : N		Leaf cyanide	
		F	P	F	P	F	P	F	P
CO ₂	1,8	16.21	0.0038	30.66	0.0005	21.61	0.0016	0.82	0.417
Date	1,8	1.09	0.3267	0	0.9636	0.47	0.5123	42.05	0.0002
Date*CO ₂	1,8	3.53	0.097	11.94	0.0086	7.39	0.0263	0.04	0.8493

Bold values are significant at $P < 0.01$.

have consistently high photosynthetic capacity even in leaves as old as 100 days (Angelov *et al.*, 1993; Cock, 1983). As one effect of [CO₂] stimulation is to enhance light limited photosynthesis in the canopy (Dermody *et al.*, 2006; Long & Drake, 1991), long lived photosynthetically active leaves would enhance cassava's whole canopy stimulation by [CO₂] when compared with other plants. Indeed, most other crops and trees show marked declines in photosynthetic capacity (i.e. lower $V_{c,max}$) of older leaves within the canopy, particularly at elevated [CO₂] (e.g., Dermody *et al.*, 2006; Takeuchi *et al.*, 2001).

Potato, sugar beet, and cassava are generically referred to as tuber crops, but in fact each of these storage organs are anatomically quite different structures, which may also contribute in unknown ways to the differences in yield responsiveness to elevated [CO₂]. Potato is a stem tuber and as such botanically is the only true tuber among the three. Both potato tuber size and number can affect the strength and duration of the sink. The sugar beet storage organ is primarily a modified taproot that stores sucrose. Typically, sugar beet forms only a single storage organ perhaps limiting the responsiveness of sugar beet sink strength and duration under increased [CO₂] growth conditions (see below). In cassava the storage roots form from secondary growth of the fibrous roots and are therefore anatomically true roots (Wheatley & Chuzel, 1993). As is the case for potato, cassava storage root size and number can affect the strength and duration of the sink.

How do our findings compare to chamber studies on tuber and root crops? The trend that growth and yield stimulation under (FACE) are similar, but muted when compared with open top chamber, and controlled environment studies at elevated [CO₂] (Ainsworth & Long, 2005; Nowak *et al.*, 2004) also appears for plants with inherently large sink to source ratios. Konjac (*Amorphophallus konjac*) is a shade dwelling deciduous C₃ aroid related to the titan arum, which forms large underground edible corms. Although it has half the photosynthetic capacity of potato, konjac biomass increased ca. 70% and corm yield over 100% in a greenhouse experiment at a [CO₂] 700 ppm when compared with 350 ppm

[CO₂] (Imai & Coleman, 1983). Imai *et al.* (1984) reported a 54% increase in total biomass, a 64% increase in harvest index, and a 150% increase in root tuber yield for cassava plants grown in a greenhouse at an elevated [CO₂] of 700 ppm. The results of the latter two studies support the notion that increases in [CO₂] beyond our elevation target of 585 ppm will further stimulate cassava yields, as plants in those experiment were grown at 700 ppm [CO₂]. Even when grown under much lower N than in our experiment (i.e. <5 Kg N ha⁻¹ vs. 150 Kg N ha⁻¹ here), cassava tuber mass increased 49% at elevated [CO₂] of 680 ppm when compared with 480 ppm in OTC's (Fernandez *et al.*, 2002).

Transient photosynthetic acclimation

At current atmospheric [CO₂], most plants function at or near the transition between Rubisco and RuBP limited photosynthesis (Long *et al.*, 2004). Theory predicts that the metabolic control of photosynthesis shifts away from Rubisco ($V_{c,max}$) and toward RuBP regeneration capacity (J_{max}) as [CO₂] increases (Long *et al.*, 2004). Consistent with our expectations and other FACE studies (Ainsworth & Long, 2005; Leakey *et al.*, 2009; Long *et al.*, 2004), cassava Rubisco carboxylation capacity ($V_{c,max@25}$) acclimated to growth at elevated [CO₂] while maintaining higher levels of photosynthesis at its growth concentration (Fig. 5A, Fig. S2). Moreover, in the elevated [CO₂] plots light saturated photosynthesis was RuBP regeneration limited throughout the growing season, consistent with the notion of a shift in the metabolic control of photosynthesis at elevated [CO₂] (Fig. 5, right panels). Decreases in $V_{c,max@25}$ at elevated [CO₂] varied across the growing season as has been reported for other crops in FACE experiments (Bernacchi *et al.*, 2005; Borjigidai *et al.*, 2006). However, we found that photosynthetic acclimation was transient in cassava, detectable only early in the season (first three measurement dates). Decreases in $V_{c,max}$ were similarly transient for cassava and potato grown at elevated [CO₂] in open top chambers (Fernandez *et al.*, 2002; see Fig. 4 in Vandermeiren *et al.*, 2002). Transient acclimation may reflect

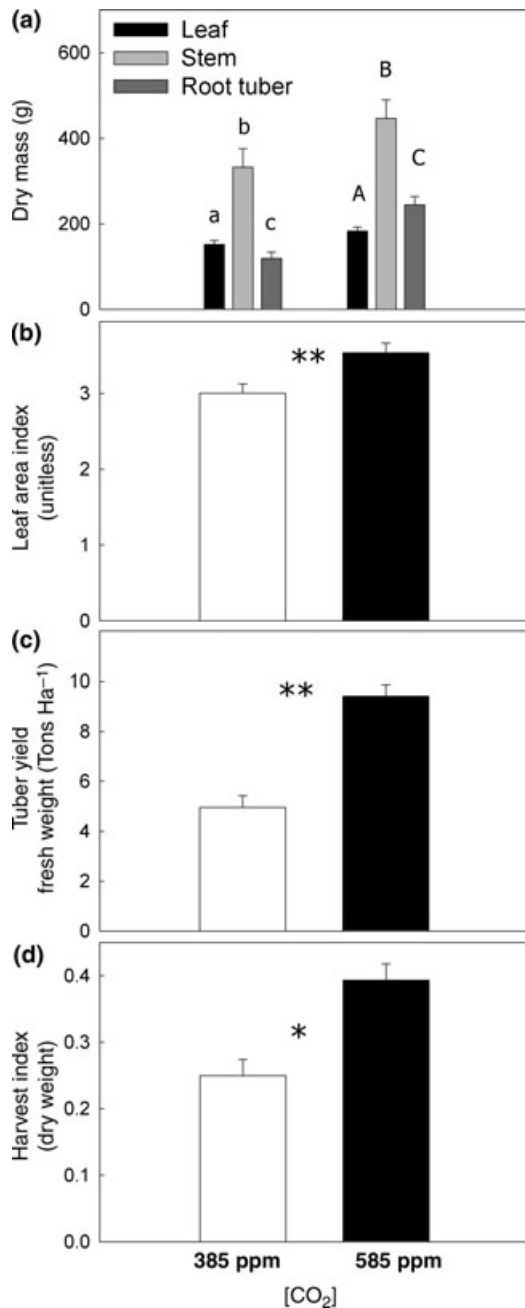


Fig. 7 Biomass allocation, leaf area index, final yield, and harvest index (root tuber biomass/above ground biomass) of cassava plants grown at ambient and elevated [CO₂]. Top panel: allocation to leaf, stem, and root tuber biomass in ambient (bars on the left) and elevated (bars on right) [CO₂]. Bars with identical letters and different cases are significantly different. Means are for $n = 4$ blocks (\pm SE). Bottom three panels: means for $n = 4$ plots in ambient (white bars) and elevated (black bars) [CO₂]. Asterisks denote significant difference between ambient and elevated [CO₂] plots (see figure 1 for P values).

lower sink strength early in the season as insufficient sink capacity increases photosynthetic acclimation to elevated [CO₂] in crops and trees (Ainsworth *et al.*,

2004; Takeuchi *et al.*, 2001). Following this line of reasoning, a lack of photosynthetic acclimation later in the season supports the notion that enhanced sink strength lowers photosynthetic acclimation (Ainsworth & Rogers, 2007) and is consistent with cassava grown in OTC's, where an absence of photosynthetic acclimation and decreases in leaf sugar and starch content later in the season coincided with an increase in growth and allocation to roots (Fernandez *et al.*, 2002).

Stomatal conductance and water use efficiency

Elevating [CO₂] improves plant instantaneous water use efficiency because stomatal conductance (g_s) functions to maximize the uptake of CO₂ (A) and minimize water loss (Cowan & Farquhar, 1977). Water use efficiency (WUE), defined here as A/g_s , was significantly greater in elevated [CO₂] plots on all measurement dates (Fig. 4, Table 2). On average, g_s is almost always lower at elevated [CO₂] (Ainsworth & Rogers, 2007); however, environmental variation modulates the response of g_s and A , and thus that of WUE, during plant growth (Ainsworth & Rogers, 2007). For instance, g_s was not lower in elevated [CO₂] plots during the first three diurnal measurement series; therefore [CO₂] induced increases in instantaneous water use efficiency early in the season were driven almost entirely by increases in A , not lower g_s at elevated [CO₂]. Stomatal conductance of well watered cassava in open top chambers was also unaffected by elevated [CO₂] (Fernandez *et al.*, 2002). In contrast, g_s was significantly lower in elevated [CO₂] plots during the last three diurnal series. Over 300 mm of precipitation fell in June and July when the first two diurnal series took place, but less than 50 mm fell in August (Fig. S3) when the last three diurnal series took place. It is likely that the larger plant canopy in elevated [CO₂] depleted the plentiful soil moisture more quickly than in ambient plots in July. Thus, in August when the canopy was at its peak and precipitation was at its lowest, decreased g_s combined with increased A to yield even larger increases in WUE in elevated [CO₂] plots. As expected, [CO₂] induced changes in g_s may work to further ameliorate impacts on WUE of cassava under intermittent mild drought at elevated [CO₂].

Potential for greater increases in cassava productivity under climate change

Maximum allocation to tuberous roots only occurs after the plant canopy reaches a peak leaf area index of ca. 3 which may not occur for several months (Alves, 2002; Cock, 1983); therefore, cassava is usually harvested after at least six months and usually 12 months of

growth (Hillocks, 2002). In Illinois, where the experiment reported here took place, May to early September proved the best growing conditions for cassava. When harvested in mid-September, cassava had just reached an optimum LAI suggesting that biomass reallocation to root tubers was reaching its peak. However, by harvesting 134 days after planting, we truncated the growing season by at least a third. Even early maturing cultivars only have 50% of their final root yield after 6 months (Howeler & Cadavid, 1983). Indeed our yields in the ambient plots are, as expected, lower than is typical for a full growing season (Rosenthal & Ort, 2012), but comparable to those of a truncated growing season (Putthacharoen *et al.*, 1998). Therefore, under the short growing season conditions here, we are likely underestimating the full season yield potential at ambient and at elevated [CO₂] by as much as 50% even as the yield ratio of ambient to elevated remains the same.

Nutrition, climate change, and the future of Cassava

The protein to carbohydrate ratio of non-legume C₃ plants usually declines, as growth at elevated [CO₂] lowers the relative protein content (Taub *et al.*, 2008). An additional potential pitfall for cassava nutrition is that growth at elevated [CO₂] is reported to increase the content of cyanide relative to proteins in cyanogenic crops (Cavagnaro *et al.*, 2011; Gleadow *et al.*, 1998), and this may be the case for cassava tubers (Miller *et al.* unpublished). We show large decreases in leaf N for cassava at elevated [CO₂] in contrast to Gleadow *et al.* (2009b) who reported increases in leaf N for cassava grown at elevated [CO₂] in pots. It is clearly evident for the field grown cassava that both total leaf N and leaf cyanide decreased in elevated [CO₂] plots (Fig. 6D), suggesting that overall plant cyanide content will likely be lower due to the dilution of N relative to carbon common to nonleguminous plant species grown at elevated [CO₂] (Leakey *et al.*, 2009).

We now have a good first level understanding of cassava's response to [CO₂] under field conditions, but there remain large gaps in our understanding of other global change impacts on cassava productivity. Average global temperatures are predicted to increase between 1 and 6 °C during this century (IPCC, 2007). The proportional enhancement of C₃ photosynthesis by elevated [CO₂] is expected to increase with temperature because both the specificity of Rubisco for CO₂ over O₂ and the ratio of solubilities of CO₂ vs. O₂ decline with increasing temperature (Long, 1991). This prediction appears to be borne out in cassava. Increasing day/night temperatures from 28 °C/21 °C to 33 °C/26 °C for chamber grown cassava at 700 ppm [CO₂] caused a

reported 232% yield stimulation compared to 150% in elevated [CO₂] without the increase in temperature (Imai *et al.*, 1984), consistent with the notion of greater stimulation by [CO₂] at higher growth temperatures. We have also shown that the photosynthetic temperature optimum of cassava is predicted to increase from 28 °C at current [CO₂] to 31 °C at 585 ppm (Rosenthal & Ort, 2012). Thus, increases in temperature and [CO₂] may have a synergistic effect on cassava productivity (Long, 1991), but this important prediction remains to be confirmed under field conditions. Finally, the effect of tropospheric ozone has on cassava productivity is completely unexplored. Ozone is the most damaging air pollutant to crops and it is likely that cassava productivity in southern Africa will be adversely affected by ozone (Rosenthal & Ort, 2012).

The relative production of root crops, particularly cassava in developing countries, is increasing at a greater rate than conventional coarse grains, such as millet and sorghum (Godfray *et al.*, 2010). Projections are that crop production will have to increase 55% by 2030 and 85% to over 100% by 2050 if we are to feed the more than 9 billion people expected by the year 2050 (Easterling *et al.*, 2007; Godfray *et al.*, 2010; Tilman *et al.*, 2011). The greatest increase in agricultural production will have to occur in developing countries with much of it occurring in Sub-Saharan Africa where the yield gaps are the greatest (Tilman *et al.*, 2011; Rosenthal & Ort, 2012). As much of the increase in African production has been driven by an increase in land recruitment to cultivation rather than productivity on a land area basis (F.A.O., 2010), the large cassava yield gap indicates that significant improvements in cassava productivity can be achieved by controlling pests and diseases and through agro-ecological or sustainable intensification (Bassett, 2010; Perfecto & Vandermeer, 2010; Tilman *et al.*, 2011). Coupled with stimulation by [CO₂] reported here, progress in closing the yield and nutritional gap for cassava grown in food insecure areas of the world has promise through combining disease resistant and biofortified cassava germplasm (Abhary *et al.*, 2011; Narayanan *et al.*, 2011; Sayre *et al.*, 2011; Welsch *et al.*, 2010) within a sustainable intensification framework. It appears that there are reasons to be optimistic that cassava is poised to substantially enhance food security under climate change.

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RAS and REM contributed equally.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Global yield trends of cassava from 1961 to 2009.

Data used to make these figures are from FAOSTAT

Figure S2. Light saturated photosynthesis (A_{1500}) of fully expanded sun leaves of cassava grown at SoyFACE at ambient (A) and elevated (E), and measured at ambient (385 ppm) and elevated (585 ppm) [CO₂] on five dates in 2010. Data are extracted from A vs. Ci curves measured in the field in a controlled environment leaf cuvette (LI-COR 6400) at the same light intensity (i.e. 1500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$). See article text for methods.

Figure S3. Comparison of mean monthly precipitation to total monthly precipitation in 2010. Open symbols are 100 year means of total monthly precipitation \pm SD for Urbana, Illinois. Filled circles are total monthly precipitation for 2010 in Urbana, Illinois. Note that June 2010 was significantly wetter than average and August was significantly drier.

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