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Seventeen years of carbon dioxide enrichment of sour orange trees: final results

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Abstract

The long-term responses of trees to elevated CO_2 are especially crucial (1) to mitigating the rate of atmospheric CO_2 increase, (2) to determining the character of future forested natural ecosystems and their spread across the landscape, and (3) to determining the productivity of future agricultural tree crops. Therefore, a long-term CO_2-enrichment experiment on sour orange trees was started in 1987, and the final results after 17 years are reported herein. Four sour orange trees (*Citrus aurantium* L.) were grown from seedling stage at 300 μmol mol^{-1} CO_2 above ambient in open-top, clear-plastic-wall chambers at Phoenix, AZ. Four control trees were similarly grown at ambient CO_2. All trees were supplied ample water and nutrients comparable with a commercial orchard. After a peak 2–4 years into the experiment, there was a productivity plateau at about a 70% enhancement of annual fruit and incremental wood production over the last several years of the experiment. When summed over the duration of the experiment, there was an overall enhancement of 70% of total biomass production. Much of the enhancement came from greater numbers of fruits produced, with no change in fruit size. Thicker trunks and branches and more branches and roots were produced, but the root/shoot ratio was unaffected. Also, there was almost no change in the elemental composition of the biomass produced, perhaps in part due to the minimal responsiveness of root-symbiotic arbuscular mycorrhizal fungi to the treatment.

Keywords: carbon dioxide, citrus, climate change, CO_2, density, global change, growth, orange, tree, yield

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Introduction

The CO_2 concentration of earth's atmosphere continues to rise, and general circulation models predict a consequent global warming and changes in precipitation patterns (IPCC, 2001). Plants in general are responsive to changing CO_2 concentrations, which portends changes in agricultural productivity around the world. At the same time, the ability of plants to absorb CO_2 during photosynthesis and then store the carbon in their tissues and/or sequester it in the soil has potential for slowing the rise of the atmospheric CO_2 concentration. The long-term responses of trees to elevated CO_2 are especially crucial (1) to mitigating the rate of atmospheric CO_2 increase, (2) to determining the character of future forested natural ecosystems and their spread across the landscape, and (3) to determining the productivity of future agricultural tree crops. This important nexus between trees and climate and future natural ecosystems and tree crop productivity led us to initiate a long-term CO_2-enrichment experiment on sour orange trees in 1987 using the open-top-chamber approach (e.g. Idso *et al*., 1991). Since then, several free-air CO_2 enrichment (FACE) experiments have also been initiated in open-field plots of tree species (e.g. Nowak *et al*., 2004; Delucia *et al*., 2005; Körner *et al*., 2005; Norby *et al*., 2005; Asshoff *et al*., 2006; Kubiske *et al*., 2006; Liberloo *et al*., 2006), as well as in sunlit controlled-temperature-and-CO_2 chambers (Medhurst *et al*., 2006), and these studies

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are yielding important information about the likely responses of forests to future elevated levels of CO_2.

However, our study was the longest such continuous-running CO_2-enrichment experiment ever conducted to this point in time, revealing significant interannual changes in response to elevated CO_2 as the trees grew from saplings and well into middle-age reproductive maturity. Unfortunately, the closure of our USDA-ARS laboratories in Phoenix, AZ, necessitated the termination of the experiment in January 2005. Herein, we report the final biomass and several other results from this long-term CO_2-enrichment experiment on sour orange trees.

Materials and methods

Eight sour orange trees (*Citrus aurantium* L.) were grown from seedling stage in four identically vented, open-top, clear-plastic-wall chambers at Phoenix, AZ (Idso *et al*., 1991). Sour orange is an ornamental tree often used for root stocks in commercial citrus orchards because of its disease and frost resistance. The trees were planted directly into the ground (Avondale loam; Kimball *et al*., 1992) in July 1987. The four chambers were constructed around pairs of trees. Initially, the chambers were 5.3 m long × 2.6 m wide × 2.0 m high. As the plants grew, the chambers were periodically enlarged until they reached 6.3 m long × 5.1 m wide × 9.0 m high. The target CO_2 concentration of the enriched chambers was 300 μmol mol^{-1} above that of the ambient chambers, and the sampling manifold was placed at about 3/4 the height of the trees. The automatic sampling/control system was described by Kimball *et al*. (1992). Except for short periods of chamber enlarging and very infrequent mechanical problems, enrichment was continuous 24 h day^{-1} every day since November 1987. The trees were fertilized and flood irrigated similar to practice in commercial orchards so as to maintain ample nutrients and soil moisture.

Every month during the course of the experiment, measurements were taken of the trunk circumferences, and these were converted to biovolumes using an allometric relationship established during years 2 and 3 of the experiment (Idso & Kimball, 1992a). Biomass was computed from the biovolumes using wood densities determined from pruned branches. From time to time over the years, we reported the progress of the experiment using such data (Idso & Kimball, 1997, 2001; Kimball & Idso, 2005), but herein the entirety of these data is presented.

In late winter or early spring each year, the fruit were harvested, counted, weighed, and in some years, subsamples were taken for determination of water content. After the trees became so large that branches were rubbing against and poking holes in the walls of the chambers, some pruning was done, generally just after the fruit harvests were completed for the particular year. The prunings were weighed and subsamples dried for biomass determinations. Except for a study during the 13th year of the experiment when fallen leaves and aborted fruits were collected and weighed (Idso *et al*., 2001), the fallen leaves and aborted fruits were allowed to remain on the soil surface and decompose. Therefore, the amounts of biomass from these sources are not included in the results reported herein, nor are fine roots or root exudates.

On January 27, 2005, CO_2 enrichment ceased, and the final biomass harvest commenced. First, to facilitate access, the plastic walls were removed. Then starting with the trunks, all the major branches were mapped and coded. Then, one by one using a reciprocating saw, the major branches were cut off at the trunk. Fruit was picked, weighed, counted, and a subsample taken for moisture content. Each whole branch was weighed on a hanging scale and its maximum length measured. Using lopping shears, all twigs with leaves (only a few had none) were cut off, placed in a basket, and weighed. Then based on stem diameter, the median 20% of the twigs were selected out, and their leaves were stripped off. The separate twigs and leaves were weighed, and leaves were counted and passed through a leaf area meter (Model LI-3100, LiCor Biosciences, Lincoln, NE, USA). These subsamples of leaves and twigs were oven dried (70 °C) and reweighed to determine water content, which was used to compute biomass of the whole twig and leaf samples. Subsamples were also taken for elemental, biochemical, and other analyses. Two 10-cm sections were cut from the basal and tip ends of each major branch, and their diameter measured. After oven drying and weighing, densities were computed assuming the samples were cylinders.

After all the major branches were removed, the trunks were marked where several cuts were to be made with a chain saw to obtain disk-shaped samples to send to various laboratories for further analyses, including a 10 cm thick one from about the 35 cm height, whose volume was later precisely determined by immersion in water. The lowest cut at about a 2-cm height was as low as possible with the chain saw. Fresh weights were measured on all the trunk sections and summed. After the volume measurements, those sections were split into many splinters to hasten drying, and then they were oven-dried at 70 °C. They required about 6 months before loss of weight ceased, after which the initial water contents were calculated and applied to the whole trunks.

Following the harvest of the trunks, about 15 cm of soil was hand shoveled out of the chambers down to

where we encountered the tops of major roots. Next, using a back-hoe, a trench was dug along the south sides of the chambers. The trench was about 2.5 m deep, 0.5 m wide, and about 1.5 m outside the chambers. Then, using high-pressure sprayers, soil was washed off the roots and down into the trench. A pump with a long hose removed excess water from the trench. We washed down to roughly 1.0 m below the original soil surface and exposed almost all the roots. A few, perhaps 5%, were deeper or outside the washed area. Similar to the branches, the major roots were cut off from the stump areas, and their azimuth angles noted. Any remaining soil was removed by washing in a tank. Fresh weights and lengths were measured. Like the branches, 10-cm lengths were cut from the basal and tip ends. Their diameters were measured, they were weighed, dried, and reweighed; and water contents and densities were calculated, again assuming cylinders. Finally, the stumps themselves were freed, remaining soil was washed off, and they were weighed. Their dry biomass was calculated assuming their water content was the same as that of the trunks.

Dried samples of leaves, twigs, the trunk, and roots were sent to a commercial laboratory (IAS Laboratories, Phoenix, AZ) for analyses of C, N, P, K, Ca, Mg, S, Na, Fe, Zn, Mg, Cu, and B. There were six leaf samples per tree taken from south and north sides at lower, middle, and top portions of the tree canopies. Three twig samples per tree were taken from lower, middle, and top portions of the canopy. Two root samples per tree were taken from south and north sides of each tree.

Soil cores (separated into 0–15 and 15–30 cm depths) were used to extract soil and fine root samples (before the destructive root system harvest described above) for the determination of the abundance of key root microbial symbionts, arbuscular mycorrhizal fungi (AMF), known to be important to the growth of the highly mycorrhiza-dependent *C. aurantium* (Jifon *et al*., 2002). Subsamples (4.0 g) of soil were used to extract extraradical hyphae of AMF using the aqueous extraction/filtration and quantification method described in Rillig *et al*. (1999). The abundance of AMF inside of roots was quantified by picking fine roots pieces (>20 cm total length) out of the soil cores, and measuring root colonization after staining with Trypan Blue (Rillig *et al*., 1999). We additionally fingerprinted the AMF community colonizing roots using terminal restriction fragment length polymorphism analysis based on the small subunit of the ribosomal RNA gene, following the protocol of Mummey *et al*. (2005). The latter analysis was based on samples frozen at the time of harvest.

The biomass and number data in Table 1 and Figs 1–3 were adjusted to correct for a flaw in our experimental design that only became apparent about half way through. The chambers were in an east-west row, and initially, they were totally separate, but with a larger gap between Chambers 3 and 4 to allow access to some greenhouses. After several years of chamber enlargement, Chambers 1–3 shared common walls. A consequence was that three enriched trees had end positions with more light, whereas only one ambient tree was in an end position. To remove bias from the data, for each parameter, the ratio of the average of the 'end' enriched trees to the value from the more shaded enriched tree was calculated, and similarly the ratio of the value from 'end' ambient tree to the average from the more shaded ambient trees was calculated. Then the values of all the more shaded trees (three ambient and one enriched) were multiplied by the average of the two ratios to make them equivalent to the trees in end positions, thereby removing the bias. As an example of the amount of the adjustment, the ratio of the mean final total biomass of the enriched trees to that of the ambient trees was 1.59 for the raw data and 1.51 for the adjusted data.

Means and standard errors in Tables 1–3 were calculated using trees as the experimental unit (i.e. $n = 4$, ignoring the pairing within chambers). The standard errors of the ratios (enriched/ambient or E/A) were calculated using the equation $\Delta r = (|D\Delta N| + |N\Delta D|)D^{-2}$, where Δ indicates the standard error, D is the ambient mean value in the denominator, N is the enriched mean value in the numerator, and the vertical bars denote absolute values. However, the statistical significance of differences among the means did account for the pairing within chambers, as determined using SAS procedure mixed with CO_2 level and chamber number as classes with chamber (co2) number defined as random. For those parameters sampled at more than one position (i.e. leaves from south and north at three heights in the canopy), position was an additional class that was a subsample within the main CO_2 treatment, as per the following where 'item' is the parameter being analyzed:

```
proc mixed;
class chamber co2 position;
model item = co2 | position/ddfm = kr;
random chamber(co2) position × chamber(co2);
run;
```

Results

Biomass and organ numbers

The sour orange trees were highly responsive to the elevated CO_2, as indicated by the final wood biomass values and the monthly trunk circumferences (Fig. 1a).

Table 1 Means, standard errors, and statistical significance of differences due to CO_2 level between the means of the final biomass of six organ classes of the sour orange trees, of several other miscellaneous response parameters, and of the cumulative sums over the 17-year experiment of five other parameters, including total cumulative biomass

	Enriched		Ambient			
Item	Mean	SE	Mean	SE	Pr. > *F*	Significance
Biomass at final harvest (spring 2005 after 17 years)						
Fruit biomass (kg tree^{-1})	32.9	2.1	10.9	0.6	0.0001	***
Leaf biomass (kg tree^{-1})	33.6	0.9	26.2	1.1	0.0024	**
Twig biomass (kg tree^{-1})	30.1	1.2	26.8	1.6	0.2607	
Branch biomass (kg tree^{-1})	124.6	6.4	78.8	5.1	0.0309	*
Trunk biomass (kg tree^{-1})	110.0	10.9	80.4	2.0	0.1616	
Stump biomass (kg tree^{-1})	41.0	2.6	26.3	0.3	0.0014	**
Large root biomass (kg tree^{-1})	40.6	1.7	27.7	2.2	0.0035	**
Total biomass (kg tree^{-1})	413.8	16.7	274.8	9.0	0.0250	*
Miscellaneous parameters at final harvest						
Above-ground biomass (kg tree^{-1})	332.3	13.8	220.9	6.9	0.0294	*
Below-ground biomass (kg tree^{-1})	81.6	4.2	53.9	2.5	0.0013	**
Root/shoot ratio	0.246	0.010	0.244	0.006	0.8952	
Number of fruits/tree	863	62	311	19	0.0182	**
Average fruit weight (g fruit^{-1})	39.2	1.4	38.3	0.5	0.5511	
Number of leaves/tree	78 300	2500	66 000	2700	0.0167	*
Leaf area/tree (m^2 tree^{-1})	249	8	223	8	0.1564	
Area per leaf (cm^2 leaf^{-1})	31.8	1.1	33.7	0.9	0.4181	
Specific leaf area (cm^2 g^{-1})	73.8	1.2	85.1	3.9	0.1945	
Number of major branches/tree	35.7	1.8	28.7	1.4	0.0209	*
Number of major roots/tree	29.3	1.3	26.0	0.7	0.0698	
Total branch length (m tree^{-1})	98.5	6.3	68.7	4.3	0.0904	
Total root length (m tree^{-1})	82.3	5.0	65.0	3.4	0.1316	
Final trunk diameter (cm)	24.4	0.2	21.0	0.3	0.0157	*
Trunk density (kg m^{-3})	703	7	697	4	0.6894	
Branch base density (kg m^{-3})	634	40	536	46	0.2852	
Branch tip density (kg m^{-3})	663	50	572	51	0.2621	
Cumulative parameters summed over duration of experiment						
Harvested fruit biomass (kg tree^{-1})	518.2	26.4	280.8	11.5	0.0002	***
Number of fruit per tree	13 840	350	7660	180	0.0001	***
Fruit size (kg fruit^{-1})	37.3	0.9	36.4	0.7	0.4693	
Biomass of prunings (kg tree^{-1})	197.7	16.0	110.8	13.7	0.0995	
Total cumulative biomass (kg tree^{-1})	1127	35	664	25	0.0148	*

*, **, and *** indicate significance at the 0.05, 0.01, and 0.001 levels of significance, respectively.

Note that in Fig. 1a, the circumference-based data have been scaled using the ratio of the final wood biomass measurements (306 and 212 kg tree^{-1} for enriched and ambient, respectively) to that of the last circumference-based data (505 and 301 kg tree^{-1}, respectively). The final harvested biomass values were only about 2/3 of the last measurements based on trunk circumferences, so the absolute values of wood production we have reported previously (e.g. Idso & Kimball, 1997, 2001; Kimball & Idso, 2005) were in error. Such a large extrapolation error is not surprising considering that the final biomasses were about 50 times greater than those in year 3. Nevertheless, the final ratio of enriched to ambient aboveground wood biomass was similar to those determined from trunk circumferences and still substantial, about a 51% enhancement (Figs 1a and 2, Table 1).

Based on the monthly trunk circumference measurements scaled using the final aboveground wood biomass values (Fig. 1a), there was considerable variation is the annual increments of wood addition (Fig. 1b). Yet it is apparent that after about year 3, the enriched trees steadily added about 8 more kg tree^{-1} than did the ambient trees. After about year 5, the trees put more biomass into their annual fruit production (Fig. 1c) than into wood (Fig. 1b). Like wood, however, there was considerable interannual variation in fruit production, but nevertheless, it is obvious that the elevated CO_2

stimulated fruit production more than it did the annual wood production, even amounting to more than a doubling in some years.

The enriched to ambient ratio of annual wood plus fruit production peaked in years 2–4 of the experiment at about 2.4 (Fig. 1d). Following the peak there was a decline through year 8. From year 8–17, however, the ratios were more or less at a plateau that corresponded with the value of the ratio at final harvest of 1.69. It is fortuitous that we were able to continue the experiment beyond year 8. Otherwise, extrapolating the years 4–8 decline, one might have concluded that the ratio would have reached 1.00 at about year 13 instead of the steady 1.69.

Focusing on the effects of elevated CO_2 on the final biomass of individual organs, the large branches, trunks, stumps, and large roots were all stimulated about 55% (Fig. 2). Leaves and twigs were somewhat lower at about 20%. Fruit production in the final year was stimulated a surprising 200% (Fig. 2). However, this high value appears to be somewhat of an aberration because during the last year the fruit production of the ambient trees dropped more than that of the enriched trees compared with the last several years of fruit production (Fig. 1c). The stimulation of fruit biomass was due entirely to the stimulation of fruit numbers, there being no significant effect of CO_2 on fruit size during the final year (Table 1).

Both total above- and belowground (stumps + roots) biomass were stimulated about 50% by elevated CO_2 (Fig. 2), and as a result, there was no significant effect on the root/shoot ratio.

The number of leaves per tree tended to increase (about 20%) due to elevated CO_2, whereas the area per leaf tended to decrease (about 10%) (Fig. 2, Table 1). Therefore, leaf area per tree tended to increase about 10%. Mean specific leaf area (leaf area per unit of mass) also tended to decrease about 13%.

The CO_2-enriched trees looked more bushy to our eyes, and this aspect was confirmed because the number of large branches per tree increased about 24%, and the total lengths of the large branches tended to be increased by about 43% (Fig. 2, Table 1). The number and total lengths of large roots tended to increase somewhat (13% and 27%, respectively) as well, but the changes lacked significance.

Diameters of the trunk disk samples increased 16% due to elevated CO_2 (Fig. 2, Table 1), consistent

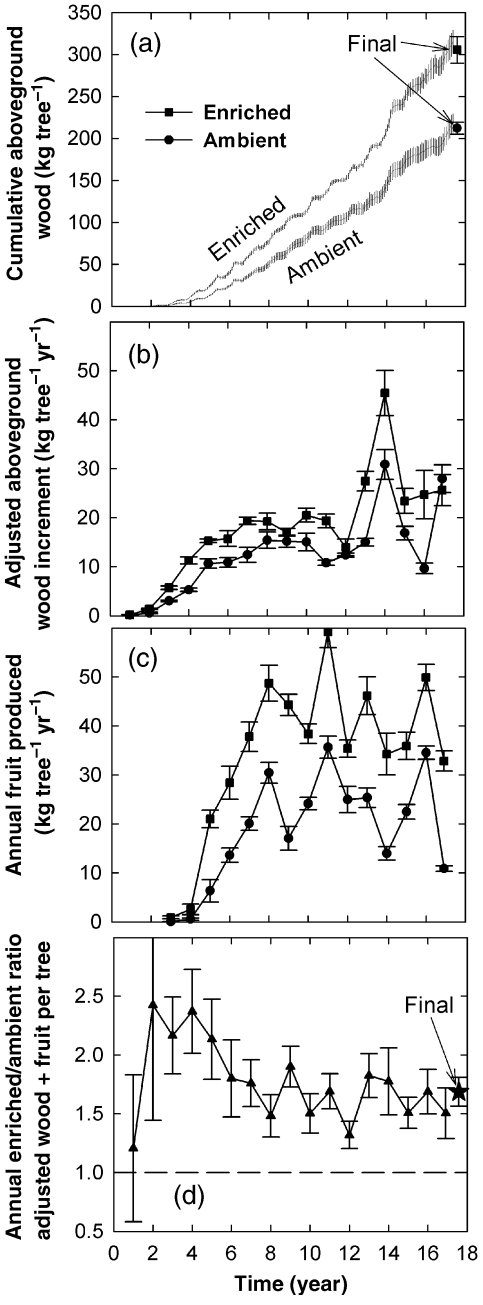

Fig. 1 (a) Final means (and standard errors) of measured aboveground wood biomass (twigs + branches + trunks + stumps) for the CO_2-enriched and ambient sour orange trees. Also shown are cumulative mean aboveground wood production and standard errors vs. time based on using monthly trunk circumference measurements in an allometric relationship from Idso & Kimball (1992a) converted from biovolume to biomass using final trunk density measurements and then scaled to the final actual biomass values. (b) Annual increments of wood production based on the trunk circumference data in (a) adjusted for the ratio of final measured aboveground wood to that estimated from the circumferences. (c) Annual fruit biomass harvests. (d) Ratios of enriched to ambient sums of adjusted aboveground wood from (b) plus the fruit biomass from (c). Also shown is the final measured ratio of enriched to ambient aboveground wood plus cumulative fruit production.

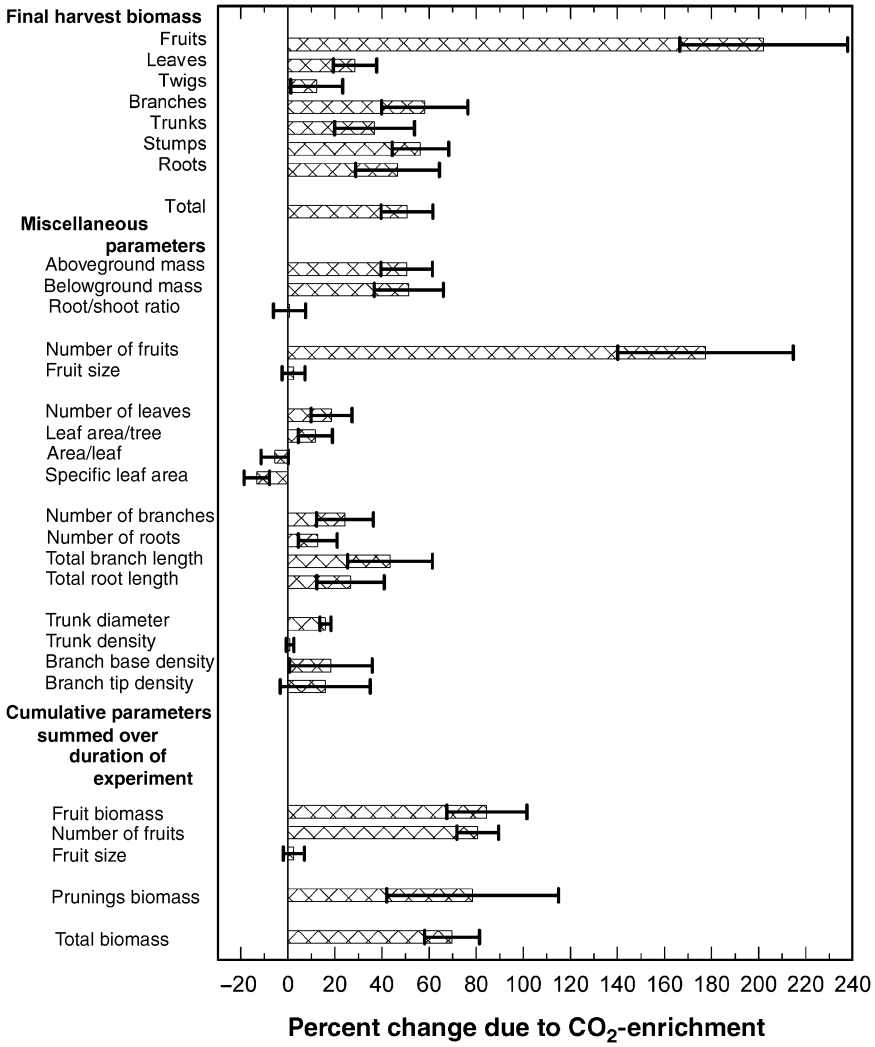

Fig. 2 Percentage changes due to CO_2 enrichment of the final biomasses of six organ classes of the sour orange trees, of several other miscellaneous response parameters, and of the cumulative sums over the 17-year experiment of five other parameters, including total cumulative biomass.

with estimates of wood biomass accumulation from circumference measurements during the course of he experiment (Fig. 1a). However, trunk density was not affected, and while the base and tip densities of the branches tended to be higher, these differences lacked statistical significance (Fig. 2, Table 1).

The cumulative amount of biomass due to fruit production over the duration of the experiment was increased 85% due to elevated CO_2 (Figs 2 and 3, Table 1). The increase was entirely from an increase in fruit number, with no increase in fruit size. Similarly, the cumulative amount of prunings biomass removed from the enriched chambers tended to be higher (78%) than that removed from the ambient chambers. Adding up the total amounts of biomass from the final harvest plus the cumulative amounts of fruit and prunings biomass removed during the course of the experiment, the total amounts of biomass produced in the CO_2-enriched and ambient treatments were 1127 and 664 kg tree^{-1}, respectively, which amounts to an overall stimulation of 70% due to elevated CO_2 (Fig. 3, Table 1).

Elemental composition

The concentrations of C, N, P, K, Ca, Mg, S, Na, Fe, Zn, Mg, Cu, and B in leaf, twig, trunk, and root samples had almost no response to the elevated CO_2 treatment (Table 2). There were some differences detected with respect to where samples of leaves and twigs were taken on the trees (Table 2 footnotes). However, the only significant changes detected due to CO_2 were: an increase in Na in the trunks, increases in Fe and Cu in the leaves, and an

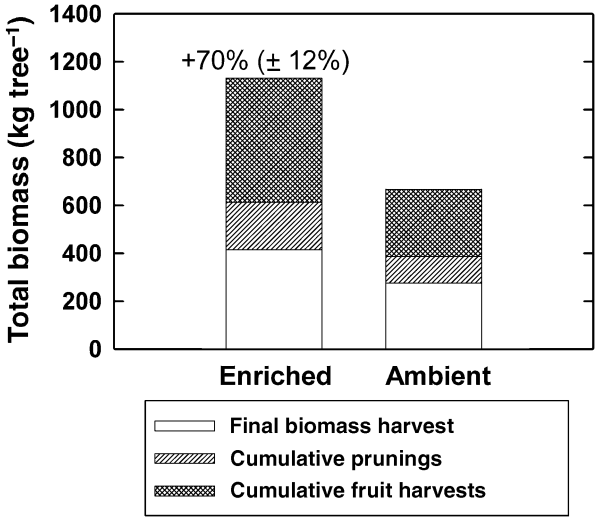

Fig. 3 Total biomass produced over the 17 years by the sour orange trees at enriched and ambient levels of CO_2 from cumulative fruit harvests, cumulative prunings, and the final biomass harvest.

increase in Zn in the roots. Of course, the lack of changes in concentration (Table 2) coupled with the large increases in biomass (Table 1) implies that there were large increases in nutrient content and in nutrient uptake from the soil under elevated CO_2 in proportion to the increases in biomass.

Arbuscular mycorrhiza

Other than a tendency for there to be longer hyphal lengths at the 15–30 cm depth ($P < 0.056$), no responses of AMF to the treatment were evident, both in the intraradical and extraradical phase (Table 3). No obvious trend was apparent in the community of AMF colonizing the roots, as represented by the number of different terminal restriction fragment sizes (corresponding to fungal ribotypes).

Discussion

An overall stimulation of 70% in total biomass production of the sour orange trees over the 17 years of this experiment due to a 300 μmol mol^{-1} increase in CO_2 (Fig. 3) is larger than generally observed for most plants, including woody species (Kimball, 1983; Poorter, 1993; Ceulemans & Mousseau, 1994; Idso & Idso, 1994; Wullschleger *et al*., 1997; Curtis & Wang, 1998; Norby *et al*., 1999; Janssens *et al*., 2000; Kimball *et al*., 2002). It is also larger than the increases in net primary production reported from forest FACE projects (Nowak *et al*., 2004; DeLucia *et al*., 2005; Körner *et al*., 2005; Norby *et al*., 2005; Asshoff *et al*., 2006; Kubiske *et al*., 2006; Liberloo *et al*., 2006). For example, Norby *et al*. (2005) report a median increase of $23 \pm 2\%$ with enrichment to 550 μmol mol^{-1} across a broad range of productivity levels. Linearly scaled to our CO_2 levels, their value would be about a 35% increase, still much below the 70% stimulation of our orange trees.

However, there are several instances of growth responses approaching that of our trees (e.g. Janssens *et al*., 2000). Focusing on citrus, Koch *et al*. (1986, 1987) obtained seedling growth increases of about 80% for a doubling of CO_2. Downton *et al*. (1987) observed about a 70% increase in productivity of 3-year-old Valencia oranges enriched with CO_2 only during the third year. Martin *et al*. (1995) observed a 87% increase in the growth of lemon at elevated CO_2 at supraoptimal temperatures, but the increase was only 21% at optimum temperatures.

Of course, one important difference between our sour orange tree experiment and the forest FACE experiments is that we fertilized our trees with soil nutrients like a commercial fruit orchard, whereas the natural forests were limited to the nitrogen available from their own soil processes, and generally, if soil nitrogen is limiting growth, then the response to elevated CO_2 of woody plants is smaller (e.g. Ceulemans & Mousseau, 1994; Cotrufo *et al*., 1998; Curtis & Wang, 1998; Kimball *et al*., 2002).

Fertilization regime might also explain the general lack of responsiveness of AMF we documented here, contrary to some previous field studies (reviewed in Rillig *et al*., 2002; Treseder, 2004). However, our results are consistent with those of Jifon *et al*. (2002); these researchers, also using high-nutrient-grown sour orange trees, did not find increases in AMF root colonization in their short-term elevated CO_2 pot experiment, reporting comparable levels of colonization. Nevertheless, in our study AMF continued to be present in comparable levels and diversity (t-RF richness) at high CO_2, with even a stimulation of the soil hyphal lengths at greater depth (Table 3). This may have been a contributing factor for the observed tree biomass increase of this mycorrhiza-dependent genotype (Jifon *et al*., 2002).

Several other mechanisms likely were also operative to cause the large stimulation of biomass due to elevated CO_2 in this long-term experiment (Figs 1–3; Table 1). Over years 2 and 3 of the study, the ratio of enriched to ambient net leaf photosynthesis was fairly steady at about 2.2, thus indicating no significant down regulation or acclimation (Idso & Kimball, 1991, 1992b) at that time. However, some acclimation appeared later, as indicated by a decline in the enhancement ratio of net photosynthesis to 1.28 in the 14th year (Adam *et al*., 2004).

Table 2 Means, standard errors, statistical significance of differences due to CO_2 between the means, and ratios and standard errors of the concentrations of thirteen elements in the leaves, twigs, trunks, and roots from the sour orange trees grown for 17 years at enriched or ambient levels of CO_2. Leaves were sampled from the south and north sides of the trees at low, middle, and high elevations, twigs were sampled at low, medium, and high elevations, and roots were sampled from south and north sides.

		Enriched		Ambient					
Element	Organ	Mean	SE	Mean	SE	Pr.>*F*	Significance	*E*/*A*	SE
C (%)	Leaves	41.75	0.06	41.71	0.09	0.787		1.001	0.004
	Twigs	44.33	0.17	44.17	0.24	0.732		1.004	0.009
	Trunks	49.80	0.49	50.15	0.32	0.740		0.993	0.016
	Roots	48.83	0.14	49.33	0.13	0.306		0.990	0.005
N (%)	Leaves	2.158	0.037	2.208	0.022	0.534		0.977	0.026
	Twigs	0.892	0.055	0.902	0.027	0.925*		0.989	0.090
	Trunks	0.328	0.021	0.368	0.010	0.138		0.891	0.082
	Roots	0.505	0.028	0.498	0.028	0.876		1.015	0.112
P (%)	Leaves	0.112	0.002	0.113	0.003	0.891		0.996	0.039
	Twigs	0.118	0.002	0.117	0.008	0.915		1.007	0.085
	Trunks	0.0038	0.0004	0.0056	0.0002	0.143		0.681	0.097
	Roots	0.017	0.003	0.015	0.002	0.672		1.139	0.333
K (%)	Leaves	0.675	0.026	0.671	0.030	0.927†		1.006	0.084
	Twigs	0.425	0.008	0.450	0.029	0.313‡		0.944	0.079
	Trunks	0.0693	0.0016	0.0750	0.0032	0.229		0.924	0.061
	Roots	0.0896	0.0135	0.0805	0.0079	0.744		1.113	0.276
Ca (%)	Leaves	5.317	0.309	5.758	0.203	0.528		0.923	0.086
	Twigs	3.083	0.155	3.708	0.212	0.256§		0.831	0.090
	Trunks	0.603	0.050	0.735	0.079	0.266		0.821	0.156
	Roots	0.764	0.121	0.716	0.028	0.826		1.066	0.210
Mg (%)	Leaves	0.342	0.011	0.353	0.010	0.448		0.968	0.060
	Twigs	0.111	0.005	0.136	0.008	0.204		0.818	0.084
	Trunks	0.00010	0.00000	0.00033	0.00023	0.374		0.308	0.213
	Roots	0.0060	0.0025	0.0063	0.0025	0.941		0.945	0.781
S (%)	Leaves	0.179	0.008	0.160	0.001	0.154¶		1.117	0.061
	Twigs	0.047	0.003	0.048	0.001	0.729		0.972	0.079
	Trunks	0.020	0.000	0.023	0.002	0.374		0.889	0.099
	Roots	0.039	0.002	0.035	0.002	0.337		1.107	0.133
Na (%)	Leaves	0.017	0.002	0.018	0.002	0.804‖		0.930	0.210
	Twigs	0.017	0.001	0.016	0.002	0.808**		1.053	0.192
	Trunks	0.180	0.010	0.125	0.010	0.014	*	1.440	0.190
	Roots	0.143	0.017	0.121	0.010	0.516		1.180	0.231
Fe (ppm)	Leaves	104.0	5.2	125.3	5.0	0.004	**	0.829	0.074
	Twigs	89.5	12.8	103.8	7.3	0.402		0.862	0.184
	Trunks	19.7	5.8	19.3	2.0	0.762		1.022	0.406
	Roots	46.9	11.3	39.5	11.9	0.691		1.187	0.642

Zn (ppm)	Leaves	18.3	1.1	18.7	0.7	0.606††		0.978	0.096
	Twigs	16.7	1.5	19.4	0.6	0.169		0.858	0.104
	Trunks	4.78	0.79	4.89	0.67	0.867		0.978	0.295
	Roots	7.56	0.87	4.34	0.30	0.039	*	1.743	0.322
Mn (ppm)	Leaves	20.88	1.48	21.29	0.74	0.871		0.980	0.103
	Twigs	7.00	0.95	7.92	0.25	0.616		0.884	0.148
	Trunks	2.32	0.05	2.15	0.06	0.090		1.080	0.051
	Roots	3.38	0.61	2.99	0.40	0.623		1.133	0.356
Cu (ppm)	Leaves	6.75	0.16	8.08	0.16	0.015	*	0.835	0.036
	Twigs	6.17	0.10	6.83	0.22	0.091		0.902	0.042
	Trunks	2.32	0.62	1.65	0.05	0.437		1.406	0.417
	Roots	2.48	0.63	2.45	0.66	0.976		1.013	0.531
B (ppm)	Leaves	199.2	17.2	202.1	7.1	0.796‡‡		0.986	0.120
	Twigs	16.75	0.75	18.08	0.32	0.086		0.926	0.058
	Trunks	5.85	0.32	5.75	0.10	0.759		1.018	0.072
	Roots	5.94	0.70	5.31	0.47	0.614		1.119	0.231

* and ** indicate significance at the 0.05 and 0.01 levels of significance, respectively.

*Twigs from the tops of the trees had significantly (Pr. > $F = 0.014$) more N than those from the bottoms.

†Leaves from the tops of the trees had significantly (Pr. > $F = 0.049$) more K than those from the bottoms.

‡Twigs from the tops of the trees had significantly (Pr. > $F = 0.012$) more K than those from the bottoms.

§Twigs from the tops of the trees had significantly (Pr. > $F = 0.034$) less Ca than those from the bottoms.

¶Leaves from the south sides of the trees had significantly (Pr. > $F = 0.009$) more S than those from the north sides.

‖Leaves from the tops of the trees had significantly (Pr. > $F = 0.032$) less Na than those from the bottoms.

**Twigs from the middle of the trees had significantly (Pr. > $F = 0.004$) less Na than those from above or below.

††Leaves from the south sides of the trees had highly significantly (Pr. > $F = 0.0007$) more Zn than those from the north sides, and higher leaves more than lower leaves.

‡‡Leaves from the south sides of the trees had significantly (Pr. > $F = 0.027$) more B than those from the north sides.

Table 3 Means, standard errors, statistical significance of differences due to CO_2 between the means, and ratios and standard errors of arbuscular mycorrhizae extraradical hyphal lengths, AMF root colonization percentages, and the number of different terminal restriction fragment sizes (t-RF) reflecting the AMF community colonizing roots from the sour orange trees grown for 17 years at enriched or ambient levels of CO_2

		Enriched		Ambient				
Item	Depth (cm)	Mean	SE	Mean	SE	Pr. > *F*	*E*/*A*	SE
Hyphal length (m)	0–15	3.41	0.74	2.87	0.31	0.526	1.187	0.386
	15–30	3.14	0.25	2.21	0.30	0.056	1.421	0.307
Colonization (%)	0–15	6.00	1.67	7.57	3.17	0.779	0.793	0.553
	15–30	9.29	4.39	8.20	3.84	0.919	1.132	1.065
t-RF number	0–15	11.3	0.9	13.8	0.9	0.189	0.818	0.113
	15–30	11.3	1.0	12.8	0.9	0.486	0.882	0.140

Statistical significance was determined using SAS procedure mixed with CO_2 level and chamber number as classes with chamber number defined as random.

Moving from the leaf to a whole canopy level, the annual productions of fruit plus wood in year 3 were 6.6 and 3.1 kg tree^{-1} for enriched and ambient trees, respectively (Idso & Kimball, 1992a) from leaf areas of 47 and 27 m^2 tree^{-1}, respectively. These data result in canopy productivity indices (CPIs; annual biomass production per leaf area, e.g. Norby *et al.*, 1999) of 0.14 and 0.12 kg yr^{-1} m^{-2}, respectively. Averaging over the last 3 years of the experiment, the annual wood plus fruit productions were 192 and 123 kg tree^{-1} (Fig. 1b and c) from leaf areas of 249 and 223 m^2 (Table 1) resulting in CPIs of 0.26 and 0.18, respectively. Thus, the CPIs were substantially higher at the end of the experiment than they were in year 3. Moreover, elevated CO_2 increased the CPI in year 3 by 23% and by 41% in years 15–17. The value of 23% is close to the mean of 12 CO_2-enrichment experiments on trees reviewed by Norby *et al.* (1999), whereas a 41% increase due to elevated CO_2 is higher than any of the previous experiments reviewed by them.

Another operative factor contributing to our large CO_2 stimulation was that the enhancement at low light within the canopy more than compensated for self-shading produced by the CO_2-induced proliferation of leaf area (Idso *et al.*, 1993b). Undoubtedly, another important aspect for the large growth response in our hot climate is that the elevated CO_2 raised the upper-limiting leaf temperature for positive net photosynthesis by approximately 7 °C, which resulted in a 75% enhancement at a leaf temperature of 31 °C, 100% enhancement at 35 °C, and 200% at 42 °C (Idso *et al.*, 1995).

Another possible mechanism for the large biomass stimulation is that elevated CO_2 decreased dark leaf respiration by 20% (Idso & Kimball, 1992b), as shown by cuvette measurements taken in the second year of the experiment, although it now appears that the cuvette technique is suspect (Amthor *et al.*, 2001). Whether elevated CO_2 directly affects dark respiration remains controversial, yet other 'dark' processes can also be affected (e.g. Bunce, 2002, 2005). The forest FACE projects (e.g. Norby *et al.*, 2005) generally enriched only during the daytime, whereas we enriched 24 h day^{-1}, and Bunce (2005), for example, found that elevated CO_2 stimulated the grain yield of soybeans by 34% with daytime only enrichment but by 62% with 24 h enrichment.

Another interesting mechanism that helps explain why the orange trees had a strong response to elevated CO_2 is that they produced three putative storage proteins in their leaves with molecular masses of 33, 31, and 21 kDa (Nie & Long, 1992; Idso *et al.*, 2001). The evergreen sour orange trees generally possess 2 years worth of leaves at any given time. In the spring, there is bud burst that produces a new cohort of branches and leaves. The new branch growth following bud burst of the enriched trees was enormous compared with that of the ambient trees, reaching a peak six times greater (Idso *et al.*, 2000). Amounts of the three proteins were generally lower in the CO_2-enriched leaves during the central part of the year, but they were higher in late fall, winter, and early spring (Idso *et al.*, 2001). The decrease from their high wintertime levels in the CO_2-enriched trees possibly provided a source of nitrogen needed to sustain the rapid spring-time branch growth. Leaves of an age greater than 2 years fall throughout the year, and during most of the year, the ratio of leaf fall from the enriched to ambient trees was steady at about 1.3 (Idso *et al.*, 2001). Surprisingly, around mid-October there was a sharp peak with the ratio reaching 2.7, indicating a significant qualitative difference in the behavior of the enriched and ambient trees. The enriched trees appeared to be reabsorbing N from second-year leaves during the process of accelerated senescence. This N was stored in the storage proteins of the first-year leaves, from which it was removed in the spring to sustain the enormous burst of new branch growth in the enriched trees.

The almost complete lack of changes in elemental composition (C, N, P, K, Ca, Mg, S, Na, Fe, Zn, Mn, Cu, and B) due to elevated CO_2 (Table 2) is rather surprising considering that at least in the case of N, it is common for elevated CO_2 to cause lower concentrations (e.g. Cotrufo *et al*., 1998; Curtis & Wang, 1998; Norby *et al*., 1999; Kimball *et al*., 2002). However, these measurements from the 17th year are mostly consistent with similar measurements made on these trees in earlier years. Gries *et al*. (1993) detected no significant changes in N, P, K, Ca, Mg, S, Na, Fe, Zn, Mn, Cu, or B in the soil or roots of the well-fertilized sour orange trees 3 years into the experiment. However, concentrations of N, K, Ca, and Mn were slightly reduced in the leaves of enriched trees. Leaves from enriched trees sampled at bimonthly intervals from years 4–7 of the experiment had 4.8% less N (as well as chlorophyll a) than those from the ambient trees (Idso *et al*., 1996). Similarly working with bimonthly leaf samples, Peñuelas *et al*. (1997) reported there were clear seasonal trends in the concentration of most elements. There were initial decreases in the leaf concentration of N and the xylem-mobile and phloem-immobile Mn, Ca, and Mg, as well as a sustained increase in B. The initial reductions of N, Ca, Mn, and Mg gradually disappeared with time from years 4–7, and as reported in Table 2, they were not present in year 17.

Although the elemental concentration data in Table 2 strongly suggest that elevated CO_2 had almost no effect on the composition of the sour orange trees, perhaps also as a consequence of the minimal to absent mycorrhizal stimulation, we should mention that some changes were detected in prior studies in addition to the three putative storage proteins already discussed (Nie & Long, 1992; Idso *et al*., 2001). During the third and fourth years of the experiment, starch content per unit of leaf area was doubled while specific leaf mass increased 10–20% (Idso *et al*., 1993a). Interestingly, at that time, the area of each leaf was also increased an average of about 10%, which contrasts with final year 17 when individual leaf areas were decreased 10% in elevated CO_2 (Table 1, Fig. 2). Soluble sugars in sun-acclimated leaves were doubled due to elevated CO_2 at 7.5 years into the experiment, whereas those in shade were unaffected (Schwanz *et al*., 1996). Whether leaves were sun- or shade-acclimated made big differences in their ascorbate and glutathione antioxidant contents and activities 7.5 years into the experiment (Schwanz *et al*., 1996), but CO_2 treatment effects were not significantly different. The activities of superoxide dismutases were similar in the sun- and shade-acclimated leaves, but they decreased in response to elevated CO_2. In contrast, elevated CO_2 caused increases in ascorbate content of the sun-acclimated leaves. Similarly, the vitamin C content of the fruit was increased 7% based on samples taken from the fourth through the 12th years of the experiment (Idso *et al*., 2002).

Conclusions

The 17 years of CO_2-enrichment at $300\,\mu mol\,mol^{-1}$ above ambient caused substantial increases in growth and productivity of the sour orange trees. Rather than a continual acclimation, instead there was a sustained enhancement of about 70% in annual fruit and incremental wood production over the last several years of the experiment and an overall enhancement of 70% when total biomass production was summed over the duration of the experiment. Much of the enhancement came from greater numbers of fruits produced, with no change in fruit size. Thicker trunks and branches and more branches and roots were produced, but the root/shoot ratio was unaffected. Also, there was almost no change in the elemental composition of the biomass produced due to elevated CO_2 – just more of it. There are several mechanisms which likely contributed to the large biomass response, which was bigger than reported from the early years of FACE forest projects. While the latter are probably more representative of the natural ecosystems in which they are being conducted, nevertheless this experiment shows that the effects of elevated CO_2 on trees can be large and sustained for many years, and it suggests that the future high CO_2 concentrations likely will stimulate citrus production.

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