# Above- and below-ground responses of C$_3$–C$_4$ species mixtures to elevated CO$_2$ and soil water availability

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## Abstract

**We evaluated the influences of CO$_2$ [Control, $\sim$370 $\mu$mol mol$^{-1}$; 200 $\mu$mol mol$^{-1}$ above ambient applied by free-air CO$_2$ enrichment (FACE)] and soil water (Wet, Dry) on above- and below-ground responses of C$_3$ (cotton, G*ossypium hirsutum*) and C$_4$ (sorghum, *Sorghum bicolor*) plants in monocultures and two density mixtures. In monocultures, CO$_2$ enrichment increased height, leaf area, above-ground biomass and reproductive output of cotton, but not sorghum, and was independent of soil water treatment. In mixtures, cotton, but not sorghum, above-ground biomass and height were generally reduced compared to monocultures, across both CO$_2$ and soil water treatments. Density did not affect individual plant responses of either cotton or sorghum across the other treatments. Total (cotton + sorghum) leaf area and above-ground biomass in low-density mixtures were similar between CO$_2$ treatments, but increased by 17–21% with FACE in high-density mixtures, due to a 121% enhancement of cotton leaf area and a 276% increase in biomass under the FACE treatment. Total root biomass in the upper 1.2 m of the soil was not influenced by CO$_2$ or by soil water in monoculture or mixtures; however, under dry conditions we observed significantly more roots at lower soil depths (> 45 cm). Sorghum roots comprised 81–85% of the total roots in the low-density mixture and 58–73% in the high-density mixture. CO$_2$-enrichment partly offset negative effects of interspecific competition on cotton in both low- and high-density mixtures by increasing above-ground biomass, with a greater relative increase in the high-density mixture. As a consequence, CO$_2$-enrichment increased total above-ground yield of the mixture at high density. Individual plant responses to CO$_2$ enrichment in global change models that evaluate mixed plant communities should be adjusted to incorporate feedbacks for interspecific competition. Future field studies in natural ecosystems should address the role that a CO$_2$-mediated increase in C$_3$ growth may have on subsequent vegetation change.**

*Keywords:* carbon dioxide, cotton, free air CO$_2$ enrichment (FACE), root responses, sorghum, stable isotopes

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## Introduction

Elevated CO$_2$ most often enhances biomass more in C$_3$ (41–44%) than C$_4$ plants (22–33%) (Poorter, 1993; Wand *et al.*, 1999). Environmental stresses (e.g. soil water, nutrient availability) generally reduce the response of C$_3$, but not C$_4$, plants to CO$_2$ (Wand *et al.*,

1999; Ward *et al.*, 1999), suggesting that $C_4$ plants will maintain their competitive advantage over $C_3$ plants in $CO_2$-enriched environments. Although some work has addressed responses of both $C_3$ and $C_4$ plants in artificial mixtures (Patterson *et al.*, 1984; Patterson, 1986; Alberto *et al.*, 1996; Ziska, 2000; Newton *et al.*, 2001) and in natural plant communities (Curtis *et al.*, 1989; Curtis *et al.*, 1990; Arp *et al.*, 1993; Hamerlynck *et al.*, 1997; Clark *et al.*, 1999; Owensby *et al.*, 1999; Morgan *et al.*, 2001), effects of elevated $CO_2$ on $C_3$ and $C_4$ plant responses have primarily been evaluated in monocultures (e.g. Craine & Reich, 2001; Lee *et al.*, 2001; Reich *et al.*, 2001).

Interestingly, doubling ambient $CO_2$ increases production of $C_3/C_4$ mixed-plant communities by only about one-half (14–17%, Mooney *et al.*, 1999; Campbell *et al.*, 2000) of that generally reported for the component monocultures (Poorter, 1993; Wand *et al.*, 1999). However, $CO_2$ enrichment increased production of a $C_3/C_4$ community in the shortgrass steppe by 26–47% during years with above average annual precipitation (Morgan *et al.*, 2001). Above-ground biomass was 23–34% greater with $CO_2$ enrichment on tallgrass prairie during dry years, but no differences occurred during wet years (Owensby *et al.*, 1999). Thus, interspecific competition may moderate the growth response of plants to $CO_2$ enrichment, but the magnitude of the effect is likely influenced by soil water conditions. Interspecific competition from either $C_3$ or $C_4$ weeds reduced vegetative growth and reproductive output of the $C_3$ crop species soybean (*Glycine max*) under $CO_2$ enrichment (Ziska, 2000).

Soil water availability is often greater with $CO_2$ enrichment (Fredeen *et al.*, 1997; Owensby *et al.*, 1997, 1999; Niklaus *et al.*, 1998; Morgan *et al.*, 2001). This indirect benefit of $CO_2$ enrichment may be particularly important in water-limited ecosystems for stimulating photosynthesis (Volk *et al.*, 2000; Derner *et al.*, 2001). Both monocultures and mixed-plant communities (Owensby *et al.*, 1993, 1999; Kimball *et al.*, 1995; Pinter *et al.*, 1996; Volk *et al.*, 2000) generally exhibit greater relative increases in plant growth under $CO_2$ enrichment when soil water is plentiful.

Free-air carbon dioxide enrichment (FACE) technology has been used at several locations throughout the world to investigate impacts of elevated $CO_2$ on natural and agroecosystems (e.g. Kimball *et al.*, 2002). There is a large reference base from previous FACE experiments using monocultures of cotton (*Gossypium hirsutum* L., a $C_3$ species) and sorghum (*Sorghum bicolor* (L.) Moench, a $C_4$ species) at the facility near Maricopa, Arizona, USA. This facility is located in a hot climate with maximum air temperatures exceeding 40 °C (Ottman *et al.*, 2001). Cotton and sorghum differed markedly in their response to $CO_2$ enrichment and to interactions between $CO_2$ and soil water. For example, a 37% increase in

biomass and 43% increase of yield were observed in cotton at elevated $CO_2$, irrespective of soil water treatment (Mauney *et al.*, 1994). In contrast, elevated $CO_2$ increased total (grain + stover) yield of sorghum by only 3% with ample soil water, but 15% when soil water was limiting (Ottman *et al.*, 2001).

However, reports of research are sparse that address the influence of $CO_2$ and soil water on responses of $C_3$ and $C_4$ plants in mixed plant communities. Therefore, in order to determine the interactive effects of $CO_2$ (Control, FACE) and water supply (Wet, Dry) on $C_3$–$C_4$ plant growth, we measured above- and below-ground responses of cotton ($C_3$) and sorghum ($C_4$) plants grown in monocultures and in two levels of mixtures. Few field $C_3$–$C_4$ experiments have been conducted during the summer in a hot climate using a controlled planting array.

## Materials and methods

### CO₂ treatments

This $CO_2$ enrichment experiment was conducted in 1999 in a field at the Maricopa Agricultural Center (MAC) of The University of Arizona, Maricopa, Arizona, USA (Ottman *et al.*, 2001). Two circular plots (25 m diameter) were randomly located in each of four replicates within a 12-ha sorghum field (Fig. 1). $CO_2$ treatments (Control or FACE) were randomly assigned to plots within each replicate. Air enriched with $CO_2$ to a nominal target level *c*. 200 $\mu$mol mol$^{-1}$ above ambient was blown into the rings designated by F1 to F4 (Fig. 1) and it exited through tri-directional jets located in vertical pipes at elevations near the top of the crop canopy. Air blowers were installed in Control plots (marked C1 to C4 in Fig. 1) to provide air movement similar to that in FACE plots. Use of these blowers was especially important at night to ensure air temperatures in FACE and Control plots were similar (Pinter *et al.*, 2000). Maximum air temperature during this experiment was 43.9 °C (Ottman *et al.*, 2001).

FACE treatments were applied continuously from the date when 50% of plants emerged (1 July) until plant maturity in the FACE-Dry plots (19 October), which was the last treatment to mature. Average daytime $CO_2$ concentrations were 566 $\mu$mol mol$^{-1}$ in the FACE plots and 373 $\mu$mol mol$^{-1}$ in Control plots. Nighttime values increased to 607 $\mu$mol mol$^{-1}$ for FACE plots and 433 $\mu$mol mol$^{-1}$ for Control plots. Thus, the daytime elevation of $CO_2$ concentration in FACE plots was 193 $\mu$mol mol$^{-1}$, and 86% of 1-min averages of $CO_2$ concentration were within 10% of the target concentration. Average contamination of Control plots with $CO_2$ from FACE plots was 7–8 $\mu$mol mol$^{-1}$ during daytime.
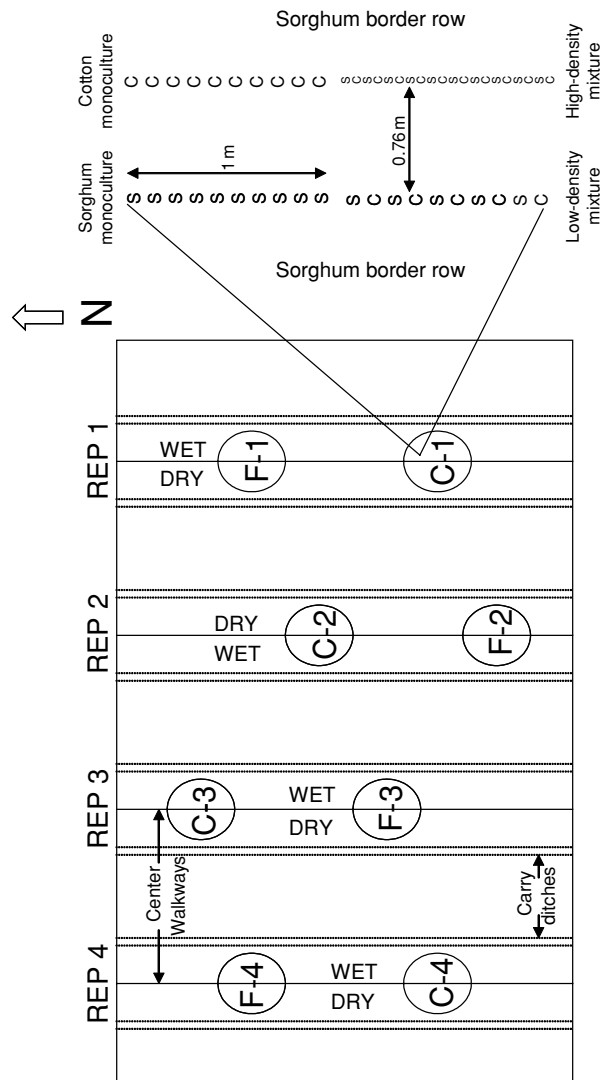
**Fig. 1** Field and plot layout plan for the 1999 FACE sorghum experiments at the University of Arizona, Maricopa Agricultural Center, Maricopa, Arizona. In the field plan, F = FACE ring with elevated $CO_2$ concentration ($566 \, \mu mol \, mol^{-1}$) and C = Control FACE ring with ambient $CO_2$ concentration ($373 \, \mu mol \, mol^{-1}$). In the plot layout plan, S = sorghum plant and C = cotton plant.

*Soil water treatments*

Each of the circular FACE and Control plots was split; half of the plot was well-watered (Wet) and half was water-stressed (Dry) (Fig. 1). Wet plots were flood-irrigated after 30% of available water in the rooting zone was depleted (Conley *et al.*, 2001; Ottman *et al.*, 2001). Plots were irrigated to replace 100% of the potential evapotranspiration since the last irrigation, adjusted for rainfall (Fox *et al.*, 1992). The Trix clay loam soil [fine-loamy, mixed (calcareous), hyperthermic Typic Torrifluvents; Post *et al.*, 1988; Kimball *et al.*, 1992) cracked when it dried. Consequently, larger amounts of

water than originally planned had to be applied at each irrigation to assure uniform coverage. Indeed, because of the relatively large minimum amounts, only two irrigations were applied to the Dry treatments (28 June and 6 August) compared to six in the Wet treatments (28 June, 23 July, 6 August, 20 August, 3 September, 17 September). Irrigation plus rainfall during 1999 totaled 1047 in Wet plots and 491 mm in Dry plots.

*Crop culture*

Sorghum stubble from a 1998 experiment was chopped on 12 January 1999, disked into the soil on 29 January, and disked a second time on 3 February. Fertilizer was applied by air on 1 June at a rate of $93 \, kg \, N \, ha^{-1}$ and $41 \, kg \, P \, ha^{-1}$. Herbicide (Dual) was applied and incorporated. Sorghum was planted on 14–15 June. Planting rate was $318\,000 \, seeds \, ha^{-1}$ ($9.97 \, kg \, ha^{-1}$; 1 seed every 4.1 cm row), and the emerged population count was $259\,500$ plants $ha^{-1}$. Fifty percent emergence date was 1 July and the FACE treatment commenced on 2 July. Weeds were hand-removed from all rings on 13 July. All plots were fertilized on 6 August with $172 \, kg \, N \, ha^{-1}$ in the irrigation water to give a total of $265 \, kg \, N \, ha^{-1}$ for the season.

*Mixture subplots*

Cotton seeds were planted on 24 June in Jiffy-Pots within a greenhouse under ambient $CO_2$ concentration at the Maricopa facility. At 2-days post-emergence (28 June), these plants were transplanted, prior to the first irrigation, to 3, 1-m-long row lengths in each $CO_2$ by water treatment combination in all rings (Fig. 1). A fourth 1-m row served as the sorghum monoculture. Five cotton plants were added to the low-density mixture of cotton and sorghum (5 plants $species^{-1} \, m^{-1}$ row length), and 10 cotton plants were added to both the cotton monoculture row (10 plants $m^{-1}$ row length) and the high-density mixture of cotton and sorghum (10 plants $species^{-1} \, m^{-1}$ row length) (Fig. 1). Sorghum plants were thinned to appropriate numbers on 17 July.

*Plant measurements*

We destructively harvested all plants within the north half meter of each row in all plots on 29 August, about 2 months after planting. This resulted in 5 plants of sorghum and cotton from each of the monoculture rows, 2–3 plants each of sorghum and cotton from the low-density rows, and 5 plants each of sorghum and cotton from the high-density rows. For sorghum plants we measured leaf area on 3 randomly chosen plants from each row. For cotton, we also recorded the number of nodes. Soil cores (4.1 cm diameter × 120 cm length) were taken between

plants within each row and divided into nine depth increments (0–10, 10–20, 20–30, 30–45, 45–60, 60–75, 75–90, 90–105, and 105–120 cm). Roots were removed from each increment by flotation in water. No distinction was made between coarse and fine roots, or between live and dead roots. We measured height of cotton and sorghum plants, to the uppermost node, and counted the number of bolls and nodes on each cotton plant of the remaining plants on 25 October. Mean internode length was calculated by dividing plant height by node number. Seed heads of sorghum plants were destructively removed and we determined seed number and seed mass per head. All above-ground tissues and roots were dried at 60 °C for 5 days prior to weighing.

Root samples from two randomly chosen replicates were finely ground using a Wig-L-Bug (model 3110-3A, Cresent Dental Mfg. Co., Lyons, Illinois, USA) and analyzed for $\delta^{13}C$ using a Carlo-Erba EA-1108 elemental analyzer interfaced with a Delta Plus (Finnigan MAT, Bremen, Germany) isotope ratio mass spectrometer operating in continuous flow mode. The isotopic composition was expressed as a $\delta^{13}C$ value where‰:

$$\delta^{13}C(‰) = \frac{\left(^{13}C/^{12}C\right)_{sample} - \left(^{13}C/^{12}C\right)_{standard}}{\left(^{13}C/^{12}C\right)_{standard}} \times 10^3$$

All $\delta^{13}C$ values were expressed relative to V-PDB (Coplen, 1995). Repeated measurements ($n = 5$) of a laboratory soil standard (Leco 502-062, Leco, St Joseph, Michigan, USA) yielded a precision of $< 0.1‰$ for $\delta^{13}C$.

The proportion of carbon derived from $C_4$ sources in root mixtures was estimated by the mass balance equation:

$$\delta^{13}C = \left(\delta^{13}C_{C_4}\right)(x) + \left(\delta^{13}C_{C_3}\right)(1-x)$$

where $\delta^{13}C$ is the $\delta^{13}C$ value of the whole sample, $\delta^{13}C_{C_4}$ is the average $\delta^{13}C$ value of the $C_4$ species (sorghum monoculture at depth 0–10 cm) for each treatment combination, $x$ is the proportion of carbon from the $C_4$ species, $\delta^{13}C_{C_3}$ is the average $\delta^{13}C$ value of the $C_3$ species (cotton monoculture at depth 0–10 cm) at each treatment combination, and $1 - x$ is the proportion of carbon from the $C_3$ species (Ludlow *et al.*, 1976; Svejcar & Boutton, 1985).

### Statistics

A split-plot design using Proc Mixed (SAS, v.8e) where $CO_2$ was the fixed effect and soil water a random effect was used to analyze above-ground plant performance in monocultures. A probability level of $\leq 10\%$ was considered significant. Where appropriate, *a posteriori* comparisons were carried out using Duncan's multiple range test. To determine if above-ground plant performance in monocultures and mixtures was influenced by $CO_2$ and soil water, we used a split–split plot design with $CO_2$ and

row (monoculture, low density or high density) as fixed effects and soil water as a random effect. Because soil depths are auto-correlated, we used soil depth as a repeated measure to analyze below-ground responses.

## Results

### Monocultures

$CO_2$ enrichment increased leaf area (86%) and above-ground biomass (85%) of cotton plants in August, and plants were 37% taller and had 5.4-fold more bolls and 20% more nodes per plant in October (Table 1). Conversely, sorghum plant responses to $CO_2$ treatments did not differ at either harvest date. Compared with the Dry soil water treatment, cotton plants in the Wet soil water treatment displayed 69% greater leaf area (August), and were 76% taller and had 41% more nodes (October), which was manifest in greater (33%) mean internode length. Soil water increased sorghum plant height by 36% in October. Root biomass (0–120 cm) was not influenced by $CO_2$ or by soil water in either the $C_3$ or $C_4$ monoculture (data not shown). Effects of $CO_2$ did not depend on soil water treatment for above- or below-ground variables for either cotton or sorghum plants.

### Mixtures

No differences were observed between low- and high-density treatments for individual plant responses of either species in the mixtures (data not shown). Leaf area (0.25 m² vs. 0.16 m², monoculture vs. mixture), above-ground mass (23.3 g vs. 17.5 g), height (37.5 cm vs. 31.9 cm) and number of nodes (21.0 vs. 17.9) of individual cotton plants were greater in monocultures than in mixtures, across $CO_2$ and soil water treatments. In contrast, number of leaves (14.2 vs. 14.1, monoculture vs. mixtures), above-ground mass (55.0 g vs. 56.3 g) and height (45.6 cm vs. 44.0 cm) of individual sorghum plants did not differ between monocultures and mixtures, across $CO_2$ and soil water treatments. However, leaf area of sorghum was reduced by 12–13% with elevated $CO_2$ in both the low- (0.41 m² vs. 0.36 m², control vs. FACE) and high-density (0.41 m² vs. 0.35 m²) mixtures, but not in the monoculture (0.36 m² vs. 0.39 m²).

Total leaf area and above-ground biomass in low density mixtures were similar between $CO_2$ treatments, but increased by 17–21% with FACE in high-density mixtures (Table 2). This increase occurred despite reductions of 13–16% in sorghum leaf area and above-ground biomass with FACE in high-density mixtures because cotton leaf area was enhanced by 121% and above-ground biomass increased in these mixtures by 276% with FACE. Root

**Table 1** Mean ($\pm$SE, $n = 4$) plant responses (m$^{-2}$) of cotton (C$_3$) and sorghum (C$_4$) in monocultures (10 plants m$^{-1}$ row length) exposed to two CO$_2$ treatments (daytime CO$_2$ concentrations: FACE, 566 $\mu$mol mol$^{-1}$; Control, 373 $\mu$mol mol$^{-1}$) and two soil water treatments (Wet and Dry). Plants were destructively harvested on 29 August and on 25 October. Percentage differences between Control and FACE treatments, and between Dry and Wet treatments are provided

| | CO$_2$ | | | | Soil water | | | |
|---|---|---|---|---|---|---|---|---|
| Variable | Control | FACE | *P*-value | % | Dry | Wet | *P*-value | % |
| *Cotton – August* | | | | | | | | |
| Leaf area (m$^2$) | 4.52 (0.84) | 8.39 (1.13) | 0.0041 | +86 | 4.81 (0.75) | 8.11 (1.32) | 0.0105 | +69 |
| Aboveground mass (g) | 432 (63) | 797 (174) | 0.0729 | +85 | 508 (158) | 721 (129) | 0.4511 | +42 |
| *Cotton – October* | | | | | | | | |
| Height (cm) | 31.6 (4.3) | 43.4 (4.5) | 0.0098 | +37 | 27.1 (2.5) | 47.8 (3.5) | 0.0074 | +76 |
| Bolls | 16 (3) | 103 (42) | 0.0719 | +544 | 42 (16) | 74 (45) | 0.5434 | +75 |
| Nodes | 503 (37) | 603 (45) | 0.0205 | +20 | 458 (24) | 647 (34) | 0.0105 | +41 |
| Internode length (cm) | 1.6 (0.3) | 1.9 (0.4) | 0.3369 | +19 | 1.5 (0.2) | 2.0 (0.2) | 0.0509 | +33 |
| *Sorghum – August* | | | | | | | | |
| Leaf area (m$^2$) | 9.58 (0.40) | 10.19 (0.31) | 0.1360 | +6 | 9.59 (0.34) | 10.17 (0.37) | 0.4052 | +6 |
| Aboveground mass (g) | 1463 (89) | 1432 (53) | 0.6869 | −2 | 1342 (66) | 1555 (66) | 0.1174 | +16 |
| *Sorghum – October* | | | | | | | | |
| Height (cm) | 44.9 (3.3) | 46.3 (2.4) | 0.4409 | +3 | 38.6 (1.2) | 52.5 (1.3) | 0.0039 | +36 |
| Seed heads | 21 (4) | 26 (4) | 0.8770 | +24 | 25 (3) | 23 (5) | 0.2087 | −8 |
| Seed mass (g) | 486 (135) | 674 (74) | 0.8007 | +39 | 483 (56) | 690 (131) | 0.1776 | +43 |

**Table 2** Total (mean $\pm$ SE, $n = 8$) leaf area (m$^2$ m$^{-2}$) and aboveground mass (g m$^{-2}$) estimated by harvesting 50% of plants in 1-m rows cotton (C$_3$) and sorghum (C$_4$) in low- (5 plants of each species m$^{-1}$ row length) and high- (10 plants of each species m$^{-1}$ row length) density mixtures on 29 August after exposure to two CO$_2$ treatments (daytime CO$_2$ concentrations: FACE, 566 $\mu$mol mol$^{-1}$; Control, 373 $\mu$mol mol$^{-1}$). Mean ($n = 8$, $\pm$ SE) total root mass (g m$^{-2}$) from 4.1 cm × 120 cm soil cores between plants within rows are also presented. Values are averaged over two soil water treatments (Wet and Dry). Percentage differences between Control and FACE treatments are provided

| | | Leaf area (m$^2$ m$^{-2}$) | | | Aboveground mass (g m$^{-2}$) | | | Root mass (g m$^{-2}$) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Mixture | Species | Control | FACE | % | Control | FACE | % | Control | FACE | % |
| Low-density | Sorghum | 5.43 (0.12) | 4.77 (0.28) | −12 | 799 (33) | 686 (61) | −14 | 117 (30) | 152 (37) | +30 |
| | Cotton | 1.17 (0.24) | 2.45 (0.53)* | +109 | 112 (25) | 285 (72)* | +154 | 28 (6) | 27 (10) | +4 |
| | Total | 6.60 (0.35) | 7.22 (0.46) | +9 | 911 (51) | 971 (61) | +7 | 145 (37) | 179 (37) | +23 |
| High-density | Sorghum | 10.64 (0.47) | 9.30 (6.89) | −13 | 1600 (99) | 1339 (109) | −16 | 151 (31) | 170 (44) | +13 |
| | Cotton | 3.01 (0.53) | 6.64 (0.98)* | +121 | 234 (45) | 881 (69)* | +276 | 54 (10) | 122 (34)* | +126 |
| | Total | 13.65 (0.89) | 15.94 (0.98)* | +17 | 1834 (121) | 2220 (134)* | +21 | 205 (40) | 292 (75) | +42 |

Asterisks indicate significant ($P < 0.10$) differences between CO$_2$ treatments.

biomass tended to be greater with FACE than Control for both low- and high-density mixtures, but differences were not significant.

Total root biomass exhibited significant row by depth interactions, with differences occurring only in the uppermost (0–10 cm) soil depth (Table 3). Root biomass in this depth was greatest in the sorghum monoculture and high-density mixture, intermediate in the low-density mixture and lowest in the cotton monoculture. For all other soil depths, root biomass was similar across

treatments. Response of root biomass to water treatments varied with depth, with greater root biomass in Wet than Dry soil water treatments in the upper two soil depths (0–10 and 10–20 cm), but the opposite occurred at lower soil depths (> 45 cm).

The relative contribution of cotton (C$_3$) and sorghum (C$_4$) to root biomass, as estimated by isotopic mass balance (see Methods), was highly variable with depth in both low- and high-density mixtures, though the general trend was for increasing contribution of C$_4$ roots with

**Table 3** Mean ($n = 16$, $\pm$ SE) root mass (g m$^{-2}$) by depth from 4.1 cm $\times$ 120 cm soil cores beneath plants within 1 m rows of cotton (C$_3$) and sorghum (C$_4$) monocultures (CM and SM, respectively, 10 plants m$^{-1}$ row length), and low- (LD, 5 plants of each species m$^{-1}$ row length) and high- (HD, 10 plants of each species m$^{-1}$ row length) density mixtures, and mean ($n = 32$, $\pm$ SE) root mass (g m$^{-2}$) for soil water treatments on 29 August following exposure to two CO$_2$ treatments (daytime CO$_2$ concentrations: FACE, 566 µmol mol$^{-1}$; Control, 373 µmol mol$^{-1}$) and two soil water treatments (Wet and Dry). Values followed by the same letter are not significantly ($P < 0.10$) different between competition treatments

| Depth | Root mass (g m$^{-2}$) | | | | | |
| | Row | | | | Soil water | |
| | SM | LD | CM | HD | Dry | Wet |
|---|---|---|---|---|---|---|
| 0–10 | 155.9 (28.6)a | 84.9 (21.9)b | 14.4 (2.4)c | 171.1 (30.8)a | 73.6 (14.5) | 143.4 (34.6)* |
| 10–20 | 30.9 (7.2)a | 21.7 (5.2)a | 13.9 (2.0)a | 42.2 (10.5)a | 24.8 (8.3) | 37.0 (11.3) |
| 20–30 | 9.2 (1.0)a | 9.4 (1.9)a | 11.8 (1.8)a | 9.5 (0.9)a | 9.0 (0.9) | 10.9 (1.1) |
| 30–45 | 9.9 (0.8)a | 11.0 (1.3)a | 11.4 (1.6)a | 14.2 (3.0)a | 11.7 (1.0) | 11.5 (1.6) |
| 45–60 | 12.3 (1.6)a | 11.1 (1.2)a | 12.5 (1.7)a | 12.8 (1.7)a | 14.8 (1.1)* | 9.5 (0.8) |
| 60–75 | 10.8 (2.1)a | 10.2 (0.2)a | 11.1 (1.5)a | 10.2 (1.3)a | 13.8 (1.2)* | 7.3 (0.7) |
| 75–90 | 7.1 (1.4)a | 8.4 (1.8)a | 10.0 (1.8)a | 7.5 (1.1)a | 11.4 (0.9)* | 5.1 (1.0) |
| 90–105 | 4.0 (0.6)a | 3.3 (1.0)a | 4.2 (0.8)a | 3.6 (0.6)a | 5.2 (0.5)* | 2.3 (0.4) |
| 105–120 | 1.5 (0.3)a | 1.7 (0.5)a | 2.6 (0.5)a | 2.4 (0.6)a | 3.0 (0.4)* | 1.1 (0.3) |
| Total | 241.7 (31.1)ab | 161.7 (25.5)bc | 91.8 (5.9)c | 273.4 (33.1)a | 167.4 (20.1) | 228.3 (36.1) |

Asterisks indicate significant differences between soil water treatments.

depth (Fig. 2). Multiplying the relative contribution of each species to root biomass by root biomass at each depth showed that sorghum comprised 81–85% of the total root biomass in the low-density mixture and 58–73% in the high-density mixtures (Table 3).

## Discussion

CO$_2$ and soil water influenced growth of C$_3$ (cotton) and C$_4$ (sorghum) plants in monocultures and mixtures. First, CO$_2$ enrichment increased above-ground growth of C$_3$, but not C$_4$, plants in monocultures, and responses to CO$_2$ were similar in both soil water treatments (Table 1). Second, CO$_2$ enrichment increased C$_3$ plant growth similarly in monocultures and mixtures, but growth responses of the C$_4$ plant with CO$_2$ enrichment were reduced in mixtures compared to monocultures. Third, elevated CO$_2$ did not affect combined C$_3$ and C$_4$ plant leaf area and biomass production in low-density mixtures, but increased both in high-density mixtures (Table 2). Fourth, total root biomass (0–120 cm) in monocultures and mixtures was not affected by elevated CO$_2$, but root biomass was distributed lower (> 45 cm) in the soil profile under Dry than Wet soil water conditions (Table 3).

### Plant responses in monocultures

Elevated CO$_2$ markedly (84–86%) enhanced leaf area and above-ground biomass of individual C$_3$, but not C$_4$

(−2 to +6%), plants in monocultures which contrasts with the general pattern of enhancing plant performance for both photosynthetic pathways (review by Wand et al., 1999). This discrepancy may be partially explained by the hot climate in which this experiment was conducted as most other field experiments have been done in more temperate climates. Previous studies using FACE at this location demonstrated a 37% increase in cotton biomass with CO$_2$ enrichment for both Wet and Dry water treatments (Mauney et al., 1994), but only a 18% increase in sorghum total (grain + stover) yield with CO$_2$ enrichment under limited water conditions and a 1% reduction under ample water conditions in the same year as this experiment (Ottman et al., 2001). The absence of CO$_2$ by soil water interactions on both C$_3$ and C$_4$ plant growth in monocultures is surprising, given that previous studies in controlled environments have demonstrated that CO$_2$ effects depend on soil water availability (Hunt et al., 1996; Ward et al., 1999). However, both root and shoot systems of a C$_4$ grass were recently determined to respond similarly to CO$_2$ irrespective of soil water availability (Derner et al., 2001). The severity of water stress, therefore, likely determines the influence of CO$_2$ on plant performance. Problems in this experiment with maintaining consistent differences in soil water availability resulting from soil cracking (see Methods) may also have contributed to the absence of significant interactions involving CO$_2$ and soil water.
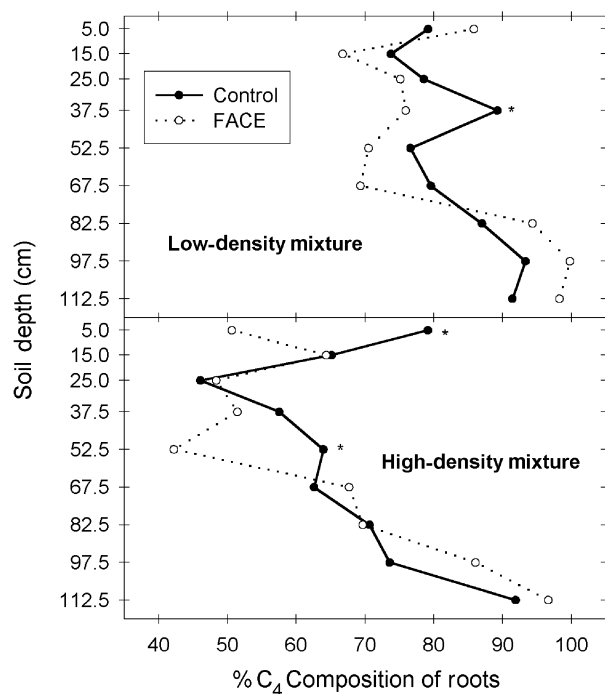
**Fig. 2** Mean ($n = 2$) % $C_4$ composition of roots, derived using $\delta^{13}C$ values of roots and mass balance equation (See Methods), by depth from 4.1 cm × 120 cm soil cores beneath plants within 1-m rows of low-density (5 plants of each species $m^{-1}$ row length) and high-density (10 plants of each species $m^{-1}$ row length) mixtures of sorghum and cotton plants on 29 August following exposure to two $CO_2$ treatments (daytime $CO_2$ concentrations: FACE, 566 $\mu$mol $mol^{-1}$; Control, 373 $\mu$mol $mol^{-1}$) and two soil water treatments (Wet and Dry).

NOTE: Sorghum monocultures were grown on these same plots in 1997 and 1998.

### Did plant responses differ in monocultures and mixtures?

Individual plant performance of the $C_3$, but not the $C_4$, plant decreased in mixtures compared to monocultures across $CO_2$ and soil water treatments. Surprisingly, plant density within mixtures did not affect growth of either $C_3$ or $C_4$ plants across other treatments. Plants generally benefit less from $CO_2$ enrichment in the presence of neighbors (du Cloux *et al.*, 1987; Ackerly & Bazzaz, 1995; Retuerto *et al.*, 1996; Wayne *et al.*, 1999), but little is known regarding relative effects of intra- and inter-specific competition on responses of individual plants to $CO_2$ enrichment under field conditions. This knowledge is required to more fully understand physiological mechanisms that influence competitive outcomes and may result in compositional shifts in plant communities.

Greater combined $C_3$ and $C_4$ leaf area and biomass production of the 20 plants in high-density mixtures with $CO_2$ enrichment occurred because enhancement of $C_3$ growth more than compensated for the reduction in

$C_4$ growth. Cotton responded similarly to $CO_2$ enrichment in monocultures and mixtures, but growth of the $C_4$ plant decreased non-significantly with $CO_2$ enrichment in mixtures compared to monocultures. Although a similar relationship existed in low-density mixtures, the magnitude of enhancement of $C_3$ plant growth was not sufficient to compensate for reduced $C_4$ performance. Parameters measured on individual $C_3$ plants did not differ statistically between low- and high-density mixtures, but there was a trend for greater leaf area and above-ground biomass of cotton plants in high-than low-density mixtures. This difference, when compounded with the greater number of plants in high-vs. low-density mixtures, was responsible for the significant effect of $CO_2$ enrichment on leaf area and above-ground biomass of the high-density mixture.

Plant composition and soil water, but not $CO_2$, influenced root biomass with differential responses in upper and lower soil depths. Root biomass in the uppermost soil depth (0–10) was two-fold greater in the sorghum monocultures and high-density mixtures than in low-density mixtures, and 11-fold more than in the cotton monocultures. Yet, root biomass was similar among the monocultures and mixtures at all other soil depths, suggesting that observed differences nearest the soil surface reflected contrasting rooting systems of the $C_3$ (taproot) and $C_4$ (fibrous and diffuse) plants. In addition, the taking of soil cores between plants resulted in an underestimation of root biomass from the $C_3$ plant because most cotton root biomass is associated with the taproot. Soil water affected allocation of roots as plants in Dry water treatments increased carbon allocation belowground to deep roots whereas in Wet treatments, belowground carbon was disproportionately allocated to shallow roots.

### Conclusions

$CO_2$ enrichment influenced above-ground responses of the $C_3$, but not the $C_4$, plant in monocultures. Surprisingly, $CO_2$ effects did not interact with soil water. Above-ground performance of individual $C_3$, but not $C_4$, plants was reduced in mixtures compared to monocultures, implying that sorghum was the superior competitor in mixtures. $CO_2$-enrichment likely partly offset negative effects of competition on cotton in both low- and high-density mixtures by increasing above-ground biomass, with a greater relative increase in the high-density mixture. As a consequence, $CO_2$-enrichment increased total above-ground biomass and leaf area of the cotton and sorghum mixture at high-density. Therefore, global change models that include individual plant responses to $CO_2$ enrichment need to incorporate the feedback of interspecific competition. There remains a critical need to

address the role that a $CO_2$-mediated increase in $C_3$ growth may have under field conditions in natural eco-systems to more fully understand $CO_2$ effects on vegeta-tion change.

## Acknowledgements

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