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Cotton Root and Rhizosphere Responses to Free-Air CO_2 Enrichment

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I. INTRODUCTION

Plant responses to above-ambient levels of CO_2 have been summarized in several excellent reviews of recent origin, each with its own special intent. Lemon (1983), Waggoner (1984), and Wittwer (1985) offer a solid background. Good syntheses of this topic have also been given by Allen (1989), Curry *et al.* (1988), Enoch and Zieslin (1988), Gifford (1988), Morison (1988), and Strain (1987). Kimball (1983a, 1983b) provides a comprehensive statistical synthesis of previous research. Strain and Cure (1985) give in-depth coverage to recent findings. Krupa and Kickert (1989) have summarized the greenhouse effect in terms of ultraviolet-B radiation, CO_2, and ozone. They underscore the need for multifactor studies. Smith and Tirpak have also provided good coverage of climate (1989a) and CO_2 (1989b) relationships to agriculture as have Adams *et al.* (1990) in a more condensed fashion using model output. In their very recent ASA Special Publication, Kimball *et al.* (1990) offer a state-of-the-art report on the impact of CO_2 on global agriculture (including a look at the greenhouse effect, trace gases, and possible influence on soils). Primary productivity in agro-ecosystems has been covered in a recent book (Goudriaan *et al.*, 1990). Especially germane to our current effort is the past research on elevated atmospheric CO_2 responses of root systems and their rhizospheres, and experimental systems used to expose test plants to CO_2. A brief discussion of these topics follows.

In their state-of-the-art report, which reviewed 184 research papers, Acock and Allen (1985) reported that for agricultural species "virtually nothing is known about root growth response to high CO_2 concentrations, other than that the root-to-shoot ratio generally increases." They recommended that since "mechanistic information on several organ-level responses to CO_2 is particularly missing, work should be initiated on the following areas: root growth, leaf net photosynthesis rates of plants grown from seed at various CO_2 concentrations, and carbon allocation relationships between photosynthetic leaves and nonphotosynthetic growing points." Since that report, some very limited experiments within a few laboratories including our own have shown that elevated atmospheric CO_2 can cause significant changes in plant root morphology (Rogers *et al.*, 1987) and physiology (Masle *et al.*, 1990), and the root system's capacity to explore soil volumes (Chaudhuri *et al.*, 1990; Chaudhuri *et al.*, 1986; Del Castillo *et al.*, 1989; Rogers *et al.*, 1987). More specifically, substantial increases in root dry weight of winter wheat [*Triticum aestivum* (L.); Chaudhuri *et al.*, 1990], sorghum [*Sorghum bicolor* (L.) Moench.; Chaudhuri *et al.*, 1986], and soybean [*Glycine max* (L.) Merr.; Del Castillo *et al.*, 1989 and Rogers *et al.*, 1987] have been

reported when these crops were grown under higher-than-normal CO_2. Chaudhuri *et al.* (1990) reported that roots of wheat plants grown under CO_2 enrichment achieved maximum rooting depth ahead of plants grown under ambient conditions. Carbon dioxide enrichment was found to increase the number of actively growing soybean roots rather than increase root elongation rate, thus implying that high CO_2-grown plants do not increase the volume of soil explored but rather explore a given volume more thoroughly (Del Castillo *et al.*, 1989). Sorghum plants grown under added CO_2 were reported to have higher root numbers and dry weights at all soil profile depths relative to the ambient CO_2 concentration (Chaudhuri *et al.*, 1986). Rogers *et al.* (1987) demonstrated that CO_2 enrichment increased root diameter in the root hair zone, length of unbranched first order laterals, and total root length and volume. A theoretical framework of the growth and carbon economy of wheat seedlings as affected by soil resistance to penetration and CO_2 level has been developed (Masle *et al.*, 1990). Idso *et al.* (1988) reported that root:shoot ratio response to CO_2 level can vary with plant species.

The next logical extension of belowground effects induced by plant response to CO_2 is the rhizosphere, the zone of microbial activity near root surfaces where root/soil interaction takes place. Perhaps attention was first focused on this topic in a 1983 statement-of-the-art by Lamborg *et al.* Elevated CO_2 may have indirect yet significant effects on microorganisms in the plant rhizosphere that are involved in nutrient transformations. Of particular interest are nitrifiers (*Nitrosomonas*, *Nitrobacter*) and phosphorus-solubilizing bacteria (*Pseudomonas*, *Bacillus*, many other genera). These bacterial groups, although not dependent upon the host plant for carbon supply, do exist in greater numbers in plant rhizospheres where they would be likely to benefit from increased root exudation should it occur under CO_2 enrichment (Norby *et al.*, 1987). Increased activity or biomass of these bacteria could ultimately enhance plant growth. Populations of nitrifying and phosphorus-solubilizing bacteria were increased in rhizospheres of yellow poplar (*Liriodendron tulipifera* L.) seedlings grown at 692 μmol mol^{-1}, although high variability precluded statistical significance (O'Neill *et al.*, 1987b). Increased availability of nutrients could thus be an indirect benefit of elevated CO_2.

Mycorrhizae may also be stimulated by increased availability of translocated photosynthate. Colonization by ectomycorrhizae (short root tips per cm fine root and total mycorrhizal short roots per plant) was increased in seedlings of white oak (*Quercus alba* L.) and shortleaf pine (*Pinus echinata* Mill.) grown under twice ambient CO_2 (O'Neill *et al.*, 1987a). Seedlings of yellow poplar grown with CO_2 enrichment, had greater total colonization per seedling, although infection per cm of root length did not differ (O'Neill and Norby, 1988). Growth, particularly of roots, was increased in all three tree species when provided additional CO_2.

In the studies mentioned above, experimental work was conducted in environmental chambers, on first year seedlings grown in pots, or in large containers grown in outdoor chambers. Therefore, many of the responses documented above must be qualified by knowledge of the many restrictions imposed by pot culture.

With respect to belowground studies of agroecosystems, the free-air carbon dioxide exposure system (FACE), which is the subject of this book, provides an excellent opportunity. First, because of the inherent high variability of crop roots, large numbers of samples are needed which require the larger sampling areas afforded by FACE systems (300-400 m^2). The bigger test areas available also allow access by sampling machines such as farm tractors equipped with coring devices. The FACE experiments offer enough test material to permit multiple investigations of biological systems, thus providing a more comprehensive understanding. And unlike chamber systems, there is virtually no modification of meteorological conditions nor of the soil profile available to plant roots.

Very little is known of how root and rhizosphere processes are affected by additional CO_2. To help fill this gap in our knowledge base we have begun work in this area. Herein, we describe the early results of a first ever study of cotton growing in an open field enriched with CO_2. An important purpose of the entire study of which we are a part was to determine and to model the response of field-grown cotton to elevated atmospheric CO_2. The specific goal of the research summarized here was to assess belowground re-

sponses. Our efforts focus on responses of the plant root system, rhizosphere, and associated edaphic factors under free-air CO_2 enrichment.

II. MATERIALS AND METHODS

A. Yazoo City, MS, 1988

In the preliminary field trials of the FACE system, cotton [*Gossypium hirsutum* (L.) 'Stoneville 506'] was planted in 1-m rows on a 7 hectare site. Located north of Yazoo City, MS, the field was bordered on the north by the South Levee of the Yazoo River and on the east by U.S. Highway 49E. The site was on the premises of the Mississippi Chemical Corporation, across from their manufacturing facilities. The Yazoo City site was originally chosen because of its proximity to an inexpensive source of CO_2.

The soil series was Morganfield silt loam: coarse-silty, mixed, nonacid, thermic Typic Udifluvents. The cotton crop and soil were managed according to recommended practices for that area. No supplemental irrigation was used. Eight study plots were established, four at nominal CO_2 concentrations of 550 μmol mol^{-1} (surrounded by 22-m FACE arrays) and four (controls) at ambient (360 μmol mol^{-1}). Plots were spaced so as to maximize distance between them, thus minimizing the chances of enriched air blowing into control plots. Free-air controlled enrichment was maintained for a period of just over 6 weeks, ending on August 31. The system, which was in its initial stages of development, supplied CO_2 during approximately 80% of daylight hours.

On September 10, prior to final harvest on October 13, fourteen replicate soil core samples were taken. Three were for soil water content, bulk density (gravimetric, oven-dry weight basis, 105 °C), and nutrient content determinations; three for rhizosphere microbiology; and eight for root density measurements. Each sample was taken from a continuous 60-cm core cut into four sections of 15 cm each. A Giddings slotted sample tube (3.8 cm I.D. with a #123 bit; Giddings Machine Co., 1985) was driven below the 60-cm depth with a 20-kg custom-made, sleeve-type post driver to obtain a core that was transferred to a stationary tray and divided in the field. Each core was carefully inspected for uniformity and retaken if a problem occurred. Core sections were cut and raked into 530 ml prelabeled Whirl-Pak plastic bags with a putty knife, the tip of which was modified to fit the tray shape. The tray was constructed from a 1-m section of 5-cm PVC pipe split in half lengthwise and attached to a wood base. Length calibration marks made core separation easier. The tray was nailed to a portable wooden table at a slight incline with one end over the table edge to facilitate raking soil samples into bags. A car whisk broom was used to clean the tray between successive samplings. Penetrometer readings were taken for the four plots (two treated and two controls) on the south side of the field (Anderson *et al.*, 1980). Eight plants from all study plots were pulled up with the taproots and accompanying lateral roots. All samples were immediately transported by truck to our laboratory in Auburn, AL, and placed in cold storage until processed. Roots were washed from cores using a Gillison's hydropneumatic elutriation system (Smucker *et al.*, 1982; Gillison's, 1990) and refrigerated in 20% ethanol at 5°C (Bohm, 1979). Samples were placed in a shallow white-bottomed pan and roots were separated from organic debris with tweezers and suction pipettes. Lengths were measured with a Comair root length scanner (Hawker de Havilland, 1985). The various parts of the whole plant samples were measured for geometric size and/or volume and dry weights (55°C) ascertained.

For rhizosphere microbiological assays, cores were placed in plastic bags, covered with ice, and shipped in an ice-chest to Oak Ridge National Laboratory by overnight mail. Upon arrival, they were refrigerated intact at 4°C until processed. Three replicates of each CO_2 × Plot × Depth core were composited for microbial assays for a total of 21 samples (cores from plot #5 were saturated with water and impossible to sieve, so were eliminated from further analysis). Soil was sieved (2 mm) and roots recovered while sieving were placed in Formalin:Ethanol:Acetic Acid (FAA) fixative for subsequent vesicular-arbuscular (VA) mycorrhizal assessment. Dehydrogenase activity was assessed for three depth increments: 0–15 cm, 15–30 cm, and 30–45 cm. Bacterial biomass was determined for the 0–15 cm increment only.

For total bacterial biomass, 1 g (fresh weight) from each sieved soil sample was placed in 99 ml 60 mM Phosphate buffer (pH 7.6) and macerated in a blender at low speed for 2 min. Additional 1 g samples were also taken at this time and placed in a 75°C oven to allow later expression of data on a dry weight basis. Nineteen ml of bacterial suspension was removed from the blender, fixed with 1 ml of filtered (0.22 µm) formalin and refrigerated at 4°C. Soil suspension smears were stained with fluorescein isothiocyanate (Babiuk and Paul, 1970) and counted with an epifluorescence microscope within 1 wk of fixation. Twenty fields on each of two soil smears were counted per sample.

For total microbial activity (dehydrogenase assay), three replicate 1 g samples were removed from sieved soil as above (with additional samples taken for dry weight conversion) and analyzed for dehydrogenase activity using the triphenyltetrazolium chloride (TTC) reduction method (Ingham *et al.*, 1985). Soil was mixed with 0.7 g freshly prepared TTC solution and incubated at room temperature in the dark for 48 h. Formazan resulting from TTC reduction was extracted with 9.3 ml methanol and solution absorbance read at 430 nm using a spectrophotometer.

For VA mycorrhizal quantification, roots fixed in FAA were stained using trypan blue stain (Phillips and Hayman, 1970) and examined for mycorrhizal infection using the grid intersect method (Giovannetti and Mosse, 1980). Intersections were scored as mycorrhizal only if internal fungal structures (arbuscules, vesicles, spores, or penetration points with internal hyphae) were present.

B. Maricopa, AZ, 1989

The 1988 site at Yazoo City, MS, was relocated to Maricopa Agricultural Center (MAC) of the University of Arizona at Maricopa, AZ, 25 miles south of Phoenix, in 1989. Cotton [*G. hirsutum* (L.) 'Delta Pine 77'] was planted April 17, 1989. Rows were on 1-meter centers with plants being thinned to 10 per meter after full emergence. The soil series was Trix clay loam: fine, loamy, mixed (calcareous), hyperthermic Typic Torrifluvents. The field was well-watered throughout the growing season by a subsurface drip irrigation system whose tubes were buried about 25 cm below each row. Recommended farming practices of the area were followed. Again, as in 1988, eight experimental plots were established, four at nominal CO_2 concentrations of 550 µmol mol^{-1} and four at ambient, 360 µmol mol^{-1}. Free-air CO_2 enrichment (FACE) was supplied over the 1989 season using four 22-m diameter circular standpipe arrays. Complexities of new installation prevented all arrays from commencing operation on the same date; three arrays began running on May 20 and one on June 10. CO_2 enrichment stopped on September 22 (with final harvest on October 16).

On 27–29 September, eight replicate sets of 15 soil sample cores were taken from the study plots, four for soil water content, bulk density (gravimetric, oven-dry weight basis, 105°C), and nutrient content determinations; and eight for root length density and root weight density measurements at crop row center and three from the interrow zone (at 0.17, 0.33, and 0.50 m from row center). Each sample was taken from a continuous 90-cm core cut into six sections of 15 cm each. Samples were taken with a Giddings sample tube (described above) pushed into the ground with a hydraulic sampler mounted on a custom-made frame (carried and powered by a 2700 kg, 55 HP farm tractor). The sampling unit was designed to allow lateral precision positioning of the probe over a two meter distance with hydraulically driven threaded rods. Once obtained, handling of soil cores and retrieval of roots was the same as in 1988. Eight plants, with taproots and attached lateral roots, from each study plot were pulled up after loosening with a drain spade. After sampling was completed, all materials were flown to our Auburn, AL, laboratory and placed in cold storage. Geometric size and dry weights (55°C) of the various plant parts were ascertained.

Statistical analyses, which were the same in both years, were based on a randomized complete block design with four blocks and two treatments (ambient air at 360 µmol mol^{-1} CO_2 and elevated CO_2 at 550 µmol mol^{-1}). All analyses were performed using the general linear models procedure (Proc GLM) of the Statistical Analysis System (SAS Institute, Inc., 1985). Single degree of freedom t tests were used to determine the significance of differences in cotton growth variables between the two [CO_2], with block X [CO_2] used

as a measure of error variance when it was significant ($P < 0.20$). In all analyses, CO_2 treatment means were considered significantly different if they differed at the $P < 0.10$ level. Probabilities were presented in tables to allow for discussion of trends.

All data from soil cores (i.e., root densities) were analyzed for differences between [CO_2] for each core segment (=soil depth) individually in the same manner described previously. The response to soil depth was not determined for any variables in either year of the experiment.

In 1989, soil cores were taken at various distances away from the center of a row of cotton plants. These data represent repeated observations in space and were analyzed using the repeated statement under GLM. Again, these data were analyzed for differences between [CO_2] for each core segment and the response to soil depth was not determined.

III. RESULTS AND DISCUSSION

A. Yazoo City, MS, 1988

Summaries for 1988 Mississippi data are presented in Tables 16-1 through 16-5. Nutritionally, the soil (Morganfield silt loam) appeared to be well-managed with respect to pH, P, K, Mg, and Ca (Table 16-1). However, a K problem with respect to plant tissue was noted during the season. Soil water content (data not shown) was similar under both the elevated CO_2 treatments (0.194 g g^{-1}) and ambient conditions (0.178 g g^{-1}). Bulk densities, as expected, were about the same for both treatments. They were quite low for the field at large (ca 1.35 g cm^{-3}) but a tillage pan was observed to occur at approximately plow depth. This was verified by penetrometer readings (Figure 16-1) which shows a soil strength (cone resistance) increase between 20 and 30 cm. The rise in strength with increasing depth probably reflects tillage practice.

Plant measurands (Tables 16-2 through 16-4) were generally not significant, however, at the 550 µmol mol^{-1} CO_2 concentration, numerous variables exhibited a tendency to increase. For aboveground measurements (Table 16-2), a statistically significant (0.05 level) increase was seen only for height, plants being 17% taller. At the elevated CO_2 level, trends of increase were observed for stem diameter ($P=0.21$) and stem dry weight ($P=0.15$).

Table 16-3 displays geometric values and counts for taproots and their associated lateral roots. Mean values for taproot length, top diameter, dry weight, and volume were numerically higher at 550 µmol mol^{-1} than at ambient ($P=0.26$, $P=0.21$, $P=0.28$, and $P=0.18$, respectively). The number of attached laterals was 20% higher with significance at 0.05 for the elevated CO_2 treatment. The decrease in bottom diameter ($P=0.15$) of taproots was associated with a shape change where the taproot thinned as it lengthened; nevertheless, overall mass and volume rose. Table 16-4 shows root length density (root length/soil core volume) and root weight density (root dry weight/soil core volume) at each depth increment. Root length density for the entire profile tended to be higher at the 550 µmol mol^{-1} treatment level ($P=0.25$). And root dry weight density rose 33% (0.10 level). We have observed a doubling of total root length in a growth chamber study of soybean seedlings when a CO_2 level of 350 µmol mol^{-1} was compared to one of 700 µmol mol^{-1} (Rogers *et al.*, 1987); an increase of 143% was seen for root dry weight. At least in the early stages of soybean development, roots responded to a greater degree than shoots. Referring to Table 16-4, significance ($P < 0.10$) was noted at two of the depths for both root length density and root dry weight density. Viewed collectively, very clear trends of increased root growth at elevated CO_2 were observed. Root length density tended to be higher at all depths for the 550 µmol mol^{-1} treatments and particularly increased in the 0-45 cm soil depth. Similarly, Chaudhuri *et al.* (1986) reported that root length increases were usually greater in the upper soil profile for sorghum grown in glass-sided boxes under CO_2 enrichment. The relationship between rooting and crop yields has not been fully elucidated and therefore the implications of enhanced rooting are still open to question. For this experiment a final yield increase of 30–45% of both lint and yield has been reported elsewhere (Evans, 1990).

Most rhizosphere microbial parameters were not significantly different due to CO_2 enrichment

TABLE 16-1
Soil Properties at Different Profile Depths for the Soil in which Cotton was Grown under Ambient Conditions and CO_2 Enrichment at Yazoo City, MS, in 1988. Mean Values for Bulk Density, pH and Four Macronutrients are Shown. N = 12.

	CO_2 Concentration										
	360 µmol mol^{-1}						550 µmol mol^{-1}				
Depth (cm)	Db§ (g cm^{-3})	pH	P	K (kg ha^{-1})	Mg	Ca	Db§ (g cm^{-3})	pH	P	K (kg ha^{-1})	Mg	Ca
0–15	1.28	6.2	99	151	539	3077	1.26	6.1	99	162	457	2643
15–30	1.40	6.0	89	144	679	2702	1.46	5.9	93	130	608	2620
30–45	1.34	6.3	87	133	717	2777	1.33	6.5	101	133	748	2870
45–60	1.33	6.4	99	128	858	3327	1.35	7.0	99	124	821	2912

§ Db = Bulk Density

TABLE 16-2
Aboveground Growth Parameters of Cotton at Yazoo City, MS, in 1988: Height, Diameter, Node Number, Leaf Number, Boll Number, Leaf Dry Weight, Stem Dry Weight, Boll Dry Weight and Top Dry Weight Under Ambient Conditions and CO_2 Enrichment. Means, Standard Errors, Probabilities and Percent Differences are Shown. N = 4.

	CO_2 Concentration			
Variable	360 µmol mol^{-1}	550 µmol mol^{-1}	Probability§	% Difference
Height (cm)	129.2 ± 1.2	151.6 ± 6.0	0.0381	17
Diameter (mm)	14.5 ± 0.4	16.3 ± 0.9	0.2115	12
Node no.	22.1 ± 1.0	23.1 ± 0.5	0.3224	5
Leaf no.	32.0 ± 9.1	40.1 ± 14.4	0.5188	25
Boll no.	15.1 ± 1.0	16.5 ± 4.1	0.7549	9
Leaf dry wt. (g)	14.7 ± 4.7	15.8 ± 6.2	0.7637	7
Stem dry wt. (g)	44.8 ± 3.2	60.5 ± 8.1	0.1522	35
Boll dry wt. (g)	63.7 ± 6.0	63.5 ± 15.9	0.9912	–0.3
Top dry wt. (g)	123.2 ± 11.1	139.8 ± 29.9	0.5835	13

§ Probability of greater F by chance.

(Table 16-5). However, there were consistent trends for both bacterial biomass and dehydrogenase activity. Bacterial populations (numbers per g dry weight of soil) were 58% greater in cores taken from the first 15 cm in the elevated CO_2 plots (P=0.17). Dehydrogenase activity (an estimator of aerobic respiration) decreased significantly (P=0.0017) with depth for both CO_2 treatments. Activity tended to be higher in soils from the elevated CO_2 samples in both the 0-15 cm and 15-30 cm increments.

Mycorrhizal infection rates were virtually identical for both treatments. The overall mean for all samples was 36.2% of root length infected. This magnitude of infection is reasonable for plants sampled towards the end of the growing season.

B. Maricopa, AZ, 1989

The 1989 Arizona data are given in Tables 16-6 through 16-9. The soil (Trix clay loam) ap-

TABLE 16-3
Taproot Parameters of Cotton at Yazoo City, MS, in 1988: Length, Top Diameter, Middle Diameter, Bottom Diameter, Total Dry Weight, Volume and Number of Attached Lateral Roots under Ambient Conditions and CO_2 Enrichment. Means, Standard Errors, Probabilities and Percent Differences are Shown. N = 4.

	CO_2 Concentration			
Variable	360 μmol mol^{-1}	550 μmol mol^{-1}	Probability§	% Difference
Taproot				
Length (cm)	19.4 ± 1.6	22.2 ± 1.4	0.2640	14
Top diameter (mm)	14.5 ± 0.4	16.3 ± 0.9	0.2115	12
Mid diameter (mm)	7.9 ± 0.6	7.4 ± 0.6	0.6868	–6
Bottom diameter (mm)	3.4 ± 0.7	2.2 ± 0.2	0.1460	–35
Dry weight (g)¶	4.8 ± 0.4	5.5 ± 0.7	0.2852	15
Volume (cm^3)	15.3 ± 1.0	18.4 ± 2.0	0.1845	20
Laterals				
No. attached	21.6 ± 0.8	25.9 ± 0.7	0.0370	20

§ Probability of greater F value by chance.
¶ Taproot weight includes attached laterals.

TABLE 16-4
Root Length Density (km m^{-3}) and Root Dry Weight Density (kg m^{-3}) of Cotton Grown under Ambient Conditions and CO_2 Enrichment at Different Soil Profile Depths at Yazoo City, MS, in 1988. Means, Standard Errors, Probabilities and Percent Differences are Shown. N = 8.

Root Length Density (km m^{-3})

	CO_2 Concentration			
Depth (cm)	360 μmol mol^{-1}	550 μmol mol^{-1}	Probability§	% Difference
0–15	45.7 ± 6.2	58.5 ± 8.9	0.0266	28
15–30	9.5 ± 0.8	28.2 ± 13.9	0.3807	197
30–45	6.5 ± 0.7	14.2 ± 3.3	0.0209	118
45–60	4.9 ± 0.8	5.8 ± 0.6	0.6059	18
Overall mean	16.7 ± 1.7	26.7 ± 6.4	0.2550	60

Root Dry Weight Density (kg m^{-3})

	CO_2 Concentration			
Depth (cm)	360 μmol mol^{-1}	550 μmol mol^{-1}	Probability§	% Difference
0–15	0.42 ± 0.08	0.44 ± 0.08	0.8071	5
15–30	0.09 ± 0.01	0.19 ± 0.06	0.2915	111
30–45	0.06 ± 0.01	0.09 ± 0.02	0.0819	50
45–60	0.03 ± 0.005	0.09 ± 0.02	0.0538	200
Overal mean	0.15 ± 0.02	0.20 ± 0.04	0.0803	33

§ Probability of greater F by chance.

TABLE 16-5
Microbial Populations of Soil Taken from Rooting Zone of Cotton Plants Grown within FACE Array at Yazoo City, MS, 1988. N =3 for 360 μmol mol^{-1}; N = 4 for 550 μmol mol^{-1}.

Assay	Depth (cm)	CO_2 Concentration 360 μmol mol^{-1}	550 μmol mol^{-1}	Probability§	% Difference
# Bacteria g^{-1} soil (× 10^{12})	0–15	1.89 ± 0.38	2.98 ± 0.75	0.17	58
μg Formazan g^{-1} soil	0–15	50.7 ± 17.7	84.7 ± 23.2	0.24	67
	15–30	3.4 ± 2.1	15.3 ± 12.2	>>0.20	350
	30–45	9.3 ± 8.4	10.9 ± 4.3	>>0.20	17
% Mycorrhizal root length	0–45	33.0 ± 9.9	39.4 ± 2.2	>>0.20	19

§ Probability of greater F value by chance.

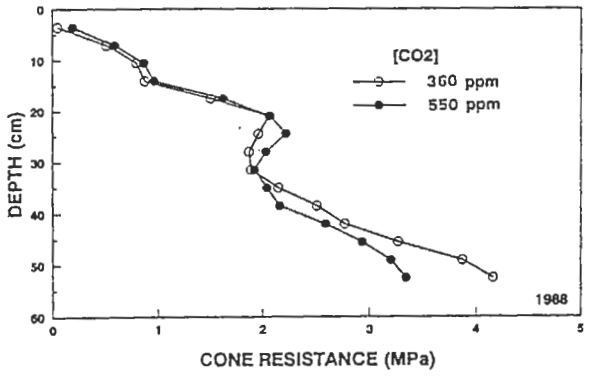

FIGURE 16-1. Penetration resistance (cone resistance; MPa) at various depths in plots with cotton grown under 360 and 550 μmol mol^{-1} CO_2 at Yazoo City, MS, 1988. N=24.

peared to be nutritionally well-managed with respect to pH, P, K, Mg, and Ca; no treatment differences were observed (Table 16-6). Soil water (data not shown) was nearly the same under both regimes (0.126 g g^{-1} for CO_2 plots and 0.132 g g^{-1} for ambient), as were bulk densities with a plow layer mean of 1.3 g cm^{-3}. A trend toward less organic matter in the high CO_2 regimes is seen (Table 16-6). No final conclusion can be drawn from this single sampling but it is interesting to note that in a new paper on the carbon economy of a planted soil as affected by elevated atmospheric CO_2, Lekkerkerk *et al.* (1990) concluded that microorganisms utilize easily decomposable root-derived material as their energy source, which was higher when plants were exposed to 700

TABLE 16-6
Soil Properties at Different Profile Depths for the Soil in which Cotton was Grown under Ambient Conditions and CO_2 Enrichment at Maricopa, AZ, in 1989. Mean Values for Bulk Density, Percent Organic Matter, pH and Four Macronutrients are Shown. N = 16.

	CO_2 Concentration													
	360 μmol mol^{-1}							550 μmol mol^{-1}						
Depth (cm)	Db§ (g cm^{-3})	OM¶ (%)	pH	P	K	Mg (kgha^{-1})	Ca	Db§ (g cm^{-3})	OM¶ %	pH	P	K	Mg (kg ha^{-1})	Ca
0–15	1.27	1.10	8.6	38	926	1120	11,199	1.34	0.95	8.5	37	898	1120	11,199
15–30	1.24	0.93	8.6	32	910	1120	11,199	1.29	0.40	8.5	30	904	1120	11,199
30–45	1.27	0.68	8.6	27	911	1120	11,199	1.33	0.52	8.6	29	885	1120	11,199
45–60	1.46	0.55	8.6	21	849	1085	11,199	1.48	0.28	8.6	17	740	1028	11,199
60–75	1.55	0.45	8.6	14	751	982	10,972	1.60	0.35	8.6	11	586	756	9,999
75–90	1.52	0.73	8.6	9	616	934	10,381	1.61	0.78	8.6	11	485	614	7,942

§ Db = Bulk Density.

¶ OM = % Organic Matter.

TABLE 16-7
Aboveground Growth Parameters of Cotton at Maricopa, AZ, in 1989: Height, Diameter, Node Number, Leaf Number, Boll Number, Leaf Dry Weight, Stem Dry Weight, Boll Dry Weight and Top Dry Weight under Ambient Conditions and CO_2 Enrichment. Means, Standard Errors, Probabilities and Percent Differences are Shown. N = 32.

	CO_2 Concentration			
Variable	360 μmol mol^{-1}	550 μmol mol^{-1}	Probability§	% Difference
Height (cm)	143.7 ± 2.6	152.6 ± 3.2	0.3091	6
Diameter (mm)	14.3 ± 0.3	17.0 ± 0.2	0.0244	19
Node no.	31.8 ± 0.6	32.1 ± 0.6	0.9293	1
Leaf no.	91.1 ± 5.7	127.5 ± 8.8	0.1305	40
Boll no.	38.4 ± 2.3	52.5 ± 2.9	0.0676	37
Leaf dry wt. (g)	27.7 ± 1.5	33.6 ± 2.3	0.4668	21
Stem dry wt. (g)	62.1 ± 3.0	92.0 ± 4.0	0.0638	48
Boll dry wt. (g)	95.8 ± 7.4	126.6 ± 9.3	0.1825	32
Top dry wt. (g)	185.6 ± 11.0	252.2 ± 13.9	0.1458	36

§ Probability of greater F value by chance.

TABLE 16-8
Taproot Parameters of Cotton at Maricopa, AZ, in 1989: Length, Top Diameter, Middle Diameter, Bottom Diameter, Total Dry Weight, Volume, Number of Attached Lateral Roots, Total Number of Laterals, Attached Lateral Root Length and Attached Lateral Root Dry Weight under Ambient Conditions and CO_2 Enrichment. Means, Standard Errors, Probabilities and Percent Differences are Shown. N = 32.

	CO_2 Concentration			
Variable	360 μmol mol^{-1}	550 μmol mol^{-1}	Probability§	% Difference
Taproot				
Length (cm)	36.4 ± 1.2	37.6 ± 1.1	0.4402	3
Top diameter (mm)	15.5 ± 0.3	19.5 ± 0.3	0.0200	26
Mid. diameter (mm)	5.7 ± 0.2	7.4 ± 0.3	0.0540	30
Bottom diameter (mm)	2.6 ± 0.1	3.4 ± 0.1	0.0001	31
Dry weight (g)¶	7.1 ± 0.3	12.9 ± 0.6	0.0140	82
Volume (cm^3)	17.6 ± 0.7	30.4 ± 1.3	0.0171	73
Laterals				
No. attached	21.9 ± 1.2	31.7 ± 1.3	0.0001	45
No. unattached	17.6 ± 1.0	21.7 ± 1.4	0.0166	23
No. total	39.5 ± 1.5	53.3 ± 2.0	0.0001	35
Attached length (cm)	1.6 ± 0.1	3.2 ± 0.2	0.0479	100
Attached dry wt. (g)	0.51 ± 0.08	1.31 ± 0.12	0.0434	157

§ Probability of greater F value by chance.
¶ Taproot weight includes laterals.

μmol mol^{-1} CO_2. This reduced the turnover of the more resistive native soil organic matter at 700 μmol mol^{-1}. Non-soluble carbon residue increased with CO_2. Perhaps such microbial phenomena relate to our data suggesting less organic matter.

Aboveground plant data showed significant increases or strong trends with CO_2 enrichment (Table 16-7). Leaf and boll numbers were both around 40% higher (P=0.13, P=0.07), and stem diameter approximately 20% higher (P=0.02) for the 550 μmol mol^{-1} plots compared to controls, but height and node number were essentially unchanged. A significant difference near the 0.05 level was noted for stem dry weight. Boll and top

TABLE 16-9
Root Length Density (km m^{-3}) and Root Dry Weight Density (kg m^{-3}) at Different Soil Profile Depths for Cotton Grown under Ambient and CO_2 Enriched Conditions at Maricopa, AZ, in 1989. Means, Standard Errors, Probabilities and Percent Differences are Shown. N = 32.

Root Length Density (km m^{-3})

Depth (cm)	CO_2 Concentration 360 μmol mol^{-1}	550 μmol mol^{-1}	Probability§	% Difference
0–15	38.0 ± 2.3	49.6 ± 2.3	0.0006	31
15–30	29.4 ± 1.8	35.5 ± 2.0	0.0334	21
30–45	15.1 ± 1.2	19.9 ± 1.2	0.0049	32
45–60	12.3 ± 1.0	11.6 ± 0.8	0.5434	–6
60–75	7.2 ± 0.5	5.8 ± 0.4	0.0179	–19
75–90	5.2 ± 0.5	4.1 ± 0.4	0.1009	–21
Mean	17.9 ± 0.8	21.1 ± 0.7	0.0033	18

Root Dry Weight Density (kg m^{-3})

Depth (cm) % Difference	CO_2 Concentration 360 μmol mol^{-1}	550 μmol mol^{-1}	Probability§	% Difference
0–15	0.31 ± 0.02	0.74 ± 0.13	0.0017	139
15–30	0.28 ± 0.04	0.48 ± 0.05	0.0032	71
30–45	0.15 ± 0.03	0.37 ± 0.08	0.0089	147
45–60	0.10 ± 0.01	0.22 ± 0.08	0.1460	120
60–75	0.06 ± 0.01	0.08 ± 0.01	0.1573	33
75–90	0.04 ± 0.01	0.06 ± 0.01	0.3970	50
Mean	0.16 ± 0.01	0.32 ± 0.03	0.0001	100

§ Probability of greater F by chance.

dry weights rose 32 and 36% (P = 0.18, P = 0.15) with elevated CO_2.

Most taproot and lateral root variables increased at 550 μmol mol^{-1} compared to 360 μmol mol^{-1}. Taproot volume and dry weight increases were 73% and 82%, respectively. All root diameter measurements were greater at the 550 μmol mol^{-1} treatment level. The total number of lateral roots was up 35% under CO_2 enrichment. In addition, CO_2 enrichment increased both lateral root length and dry weight by 100 and 157%. Appreciable differences (significant at four depths) in root length density (Table 16-9) were seen in the upper layers of the soil profile, with an average increase of 18% for the whole profile. We have no clear explanation of the reduction at greater depths, but it could be related to the onset of end-of-season senescence phenomenon. Experimental protocol did not allow us to enter the field earlier because the root and soil sampling techniques were highly intrusive and destructive.

Root dry weight density for the whole profile showed a 100% increase due to CO_2 enrichment (Table 16-9); significance occurred at the first three depths (P < 0.01) and was near the 0.1 level for the next two. The sparseness of roots deeper in the soil could increase sampling variability. In general, vertical distribution patterns are similar, but densities have a tendency to be increased by added CO_2. To provide some idea of root patterns across rows, one set of core samples was pulled from each plot at a right angle to row direction. Figure 16-2 shows this horizontal distribution of roots at four positions from row center (0.00 m) to

the middle of the interrow space (0.50 m). Patterns are very much alike without appreciable differences; the overlap of roots from adjacent rows may account for the similarity. The low sample number prohibited statistical separation, but a snapshot of the across-row profile is provided.

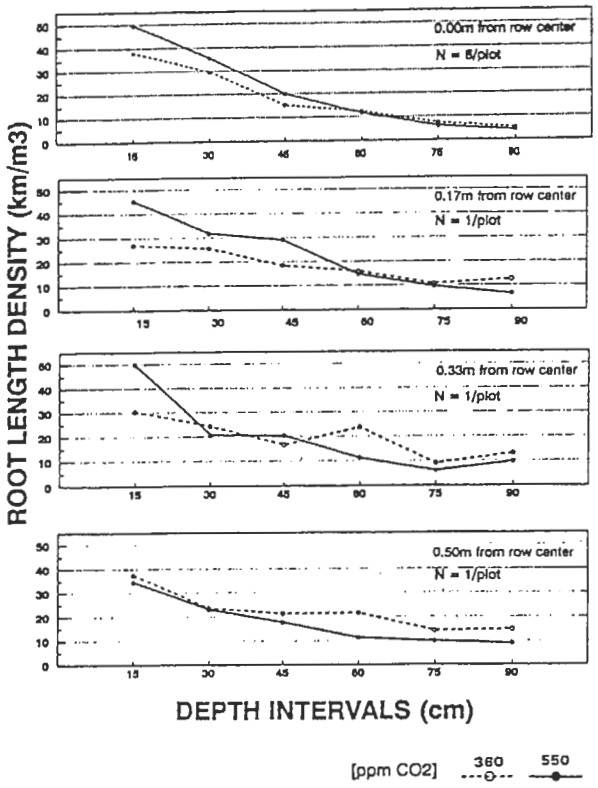

FIGURE 16-2. Mean root length density (km m^{-3}) of cotton grown under 360 and 550 µmol mol^{-1} CO_2 different soil profile depths and four positions from row center to the middle of the interrow space at Maricopa, AZ, in 1989.

Whole-field root:shoot ratios are given in Table 16-10 for both 1988 and 1989. Ratios were very close for both 360 and 550 µmol mol^{-1} CO_2 at the Mississippi site where conditions were mild with high rainfall and humidity. For the desert site in Arizona, a marked increase in root:shoot ratio was observed for the high CO_2 treatment. While it should be noted that the 1988 exposure was shorter than that for 1989, the higher percentage of total biomass allocated to roots in the arid climate suggest a possible stress amelioration by elevated CO_2. Such a strategy could reduce overall root resistance to soil water uptake.

IV. CONCLUSION

Data from the field presented here clearly suggest that elevated atmospheric CO_2 stimulates cotton root proliferation. It may be inferred that other belowground processes impacted by root proliferation could also shift. Both root length density and root weight density appear to increase, especially in the upper layers of the soil profile. Enhanced growth of cotton roots resulted in a more thorough exploration of the soil volume occupied, possibly increasing water and nutrient availability. Increased rooting induced by CO_2 enrichment could become very important for seedling establishment, especially for a crop under stress. For agronomic conditions where water and nutrients are essentially unlimited, denser root patterns may have less significance than under the limiting circumstances of more primitive or sustainable agricultural systems, or in natural ecosystems. A more thorough understanding of the implications of CO_2-enhanced root growth must await further experimentation.

Even 6 weeks of exposure to CO_2 enrichment can affect belowground structure and function. Although the rhizosphere *per se* was not sampled, increased bacterial populations and microbial activity are indicative of a trend toward a plant-mediated stimulatory effect of elevated CO_2. Mycorrhizae (per unit root length) were not significantly increased. However, greater root length density suggests that total plant mycorrhization and thus the ability to exploit soil resources were increased. Both rhizosphere and plant responses to CO_2 enrichment are heavily dependent upon seasonality, upon plant developmental stages, and upon cultural practices. A thorough understanding of the responses of the belowground system will require sampling over an extended period of time. These data clearly indicate the need for further investigation of the belowground system of plants exposed to elevated CO_2 at FACE facilities.

TABLE 16-10
Mean Root:Shoot Ratios of Cotton Grown under Ambient Conditions and CO_2 Enrichment at Yazoo City, MS, 1988, and at Maricopa, AZ, 1989. Means and Percent Differences are Shown. N = 4.

		CO_2 Concentration		
Date	Location	360 μmol mol^{-1}	550 μmol mol^{-1}	% Difference
1988	Yazoo City, MS	0.112	0.127	13
1989	Maricopa, AZ	0.114	0.168	47

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