

Effects of free-air CO₂ enrichment on microbial populations in the rhizosphere and phyllosphere of cotton

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Abstract

Cotton (*Gossypium hirsutum* L.) plants were exposed to free-air CO₂ enriched (FACE = 550 $\mu\text{mol mol}^{-1}$) or ambient (CONTROL = 370 $\mu\text{mol mol}^{-1}$) levels of atmospheric CO₂ and to wet (100% of evapotranspiration replaced) or dry (67% of ET replaced) soil water content treatments. Foliar, soil and root samples were collected in June and August 1991 to determine the effects of elevated CO₂ on selected groups of phyllosphere and rhizosphere microorganisms. Foliage and rhizosphere soil were analyzed for bacteria and/or fungi using dilution plating. Mycorrhizal colonization of cotton roots was assessed. Root-zone soil was analyzed for populations of nematodes, microarthropods and *Rhizoctonia* using various extraction methods. A dehydrogenase assay for total microbial respiration and a bioassay for cotton root infecting organisms were also conducted using root-zone soil. Populations of fungi on cotton leaves varied, by genera, in response to CO₂ enrichment, but none was affected by soil water content treatments; populations of foliar bacteria were not affected by either CO₂ or soil water content treatments. In August, higher total numbers of rhizosphere fungi were found under the wet compared with the dry soil water treatment, but differences related to CO₂ were not detected. There was a trend for infestation by *Rhizoctonia solani* to be higher under FACE in the August sample, but the soil bioassay demonstrated no increase in damping-off potential. There was a significant interaction between CO₂ concentration and soil water content for populations of saprophagous nematodes; populations were different between the CO₂ levels in the dry soil treatment only, with higher numbers under FACE. Microarthropod numbers were low; however, there was a trend for Collembola populations to be higher under FACE in the August sample and more fungi were isolated from Collembola in June. Total microbial activity was higher under FACE at both sample dates. Effects of elevated atmospheric CO₂ on plant-

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microbe interactions could have profound influence on the productivity of agro-ecosystems, and deserve further research.

1. Introduction

The global increase in atmospheric CO₂ is an established phenomenon (Keeling et al., 1989) and the concentration of CO₂ in the atmosphere may double during the next century (Bolin, 1986). Carbon dioxide is an essential compound for plant life and the Earth's vegetation will continue to be affected by increasing levels of CO₂. The manner in which increases in atmospheric CO₂ will affect the major diseases of the world's crops is largely unknown but may result in positive or negative impacts on crop health and productivity. This is of vital importance in that each year billions of dollars in crop yield are lost to plant diseases and millions more are spent controlling disease organisms.

Generalities regarding effects of CO₂ on host–pathogen interactions can be theorized using knowledge of ecophysiological differences among pathosystems. Elevated CO₂ generally promotes plant growth (Kimball, 1983) and, as plant structure is modified, the affected plants may tolerate higher levels of infection without subsequent reductions in yield. Elevated CO₂ induced changes in plant morphology may lead to alterations in microclimate which may have positive or negative impacts on disease incidence and severity. Plants which are vigorous are better able to resist infection from weak pathogens, such as facultative saprophytes, resulting in lower disease incidence and severity. However, larger plants provide more surface area for infection, and diseases (particularly those caused by obligate parasites) may increase in incidence and severity.

Elevated CO₂ may benefit plant health and productivity by altering the morphology and physiology of plants to the detriment of pathogenic microbes. Leaves of plants grown under elevated CO₂ can have more waxes and extra layers of epidermal cells (Thomas and Harvey, 1983) and lower concentrations of nitrogen and altered C:N ratios (Mellilo, 1983), all of which may lead to lower levels of foliar disease. Because photosynthetic processes increase under elevated CO₂, plants may be able to produce more defense related compounds such as phenolics (Mellilo, 1983) for restricting microbial infection. However, starch and sugar content of plant leaves also have been shown to increase under elevated CO₂ (Mauney et al., 1979; Yelle et al., 1989; Hendrix, 1992), which may provide pathogenic microbes additional substrate for increased growth and reproduction. Thompson et al. (1993) related lower powdery mildew (*Erysiphe graminis*) infection of wheat seedlings under elevated CO₂ to reductions in leaf nitrogen; he also reported that effects of CO₂ on this pathosystem were influenced by the water status of wheat seedlings. G.B. Thompson (personal communication, 1994) related lower severity of a foliar rust disease of a C₃ sedge under elevated CO₂ to reductions in leaf nitrogen concentration, but attributed an increase in foliar disease severity (fungus unknown) under elevated CO₂ for a C₄ grass to increased leaf water content.

Elevated CO₂ may also be advantageous to plant growth and health by enhancing the activities of beneficial microorganisms such as nitrogen-fixing bacteria and mycorrhizal fungi (Luxmoore, 1981; Lamborg et al., 1983), and bacterial rhizosphere colonizers (Kloepper et al., 1991). Ectomycorrhizae per cm of root of shortleaf pine (Norby et al., 1987; O'Neill et al., 1987) and percent ectomycorrhizal root apices of white oak (O'Neill et al., 1987) were significantly increased under elevated CO₂. Although elevated atmospheric CO₂ has been shown to increase nodule activity (Hardy and Havelka, 1973; Finn and Brun, 1982; Norby, 1987; Arnone and Gordon, 1990) and other plant associated responses because of nitrogen-fixing bacteria, the effects of CO₂ on the bacteria responsible for nitrogen fixation and on plant growth promoting bacterial rhizosphere colonizers have not been investigated.

Rhizosphere deposition has important implications for the development of rhizosphere biota (Curl and Truelove, 1986; Kloepper et al., 1991) which includes organisms responsible for disease suppression; hence, alteration in rhizodeposition as a result of elevated CO₂ may also impact beneficial and pathogenic microbes. The quantity of carbon in plant root exudates is known to increase under elevated CO₂ (Norby et al., 1987; Lekkerkerk et al., 1990) and it has been hypothesized that increased carbon in the soil, with accompanying decreases in nitrogen and phosphorus because of increased utilization by larger plants, will increase associations of plants with beneficial soil microorganisms (Luxmoore, 1981; Lamborg et al., 1983). However, populations of pathogenic microorganisms may also be stimulated by increased carbon from root exudation.

Changes in atmospheric CO₂ will elicit complex changes in plant–microbe interactions and in plant diseases which will vary depending upon the host, the microorganism, and the environmental factors which may be altered by an elevated CO₂ atmosphere. However, little is known concerning this vital aspect of plant health. The purpose of this study was to assess the effects of free-air CO₂ enrichment and its interaction with soil water content, on populations of selected groups of phyllosphere and rhizosphere microorganisms; the free-air CO₂ enrichment system provided a unique opportunity to assess these effects on field-grown plants without the climatic influences often associated with the use of chambers.

2. Materials and methods

2.1. Study site

The field site was located at the University of Arizona's Maricopa Agricultural Center at Maricopa, AZ. The soil was a reclaimed Trix clay loam (fine-loamy, mixed (calcareous), hyperthermic Typic Torrifuvents). Following chisel plowing, cotton seed (Deltapine 77) was sown on a 1 m row spacing on 16 April 1991. Cotton plants were thinned to 10 per meter-row and were grown according to recommended farming practices for the area. The study design was a split plot arranged as a randomized complete block with four blocks. Whole-plot treatments comprised free-air CO₂ enrichment (FACE $\approx 550 \mu\text{mol CO}_2 \text{ mol}^{-1}$) and ambient air (CONTROL ≈ 370

$\mu\text{mol CO}_2 \text{ mol}^{-1}$). Carbon dioxide treatments were achieved through use of a free-air carbon dioxide enrichment (FACE) system (Lewin et al., 1994) and were initiated on 26 April when seed had reached 50% emergence. Split-plot treatments comprised wet (100% of evapotranspiration replaced) or dry (67% of evapotranspiration replaced) soil water content. Soil water treatments were applied through use of a subsurface drip irrigation system and were initiated on May 20.

2.2. Sampling

Samples were collected in June and August 1991. The uppermost, fully expanded, south-facing leaf was collected from 20 individual cotton plants in each split plot. Leaves were placed in plastic bags, packed in a cooler with ice, and immediately express-mailed to Auburn University for assessment of phyllosphere microbial populations (fungi, bacteria and actinomycetes).

Three cores per split plot were taken from directly beside randomly selected plants with a Giddings steel sample tube (3.8 cm I.D. with a #123 bit; Giddings Machine Co., Fort Collins, CO, USA) lined with a thin-walled butyrate tube. The steel sample tube was driven below a 30 cm depth with a sleeve-type post driver and extracted by hand. Cores within the plastic tube liners (which were capped) were packed in ice, transported within 2 days to the National Soil Dynamics Laboratory (NSDL), and processed within 24 h for assessment of soil microarthropod populations.

Roots and root-zone soil were collected from eight plants in each split plot. The plant was cut at the groundline and the blade of a drain spade was pushed into the soil 10–15 cm from the stem on two sides of each plant. The spade blades were gently lifted and soil containing the taproot with attached laterals was removed and placed in a plastic bag. Bags were packed in ice, transported within 2 days to NSDL, and processed within 24 h. Rhizosphere soil, that which was adhering to roots, was carefully removed from the root surfaces and used for assessment of rhizosphere microbial populations. Lateral roots were removed for assessment of vesicular–arbuscular mycorrhizae. Root-zone soil was assessed for populations of nematodes, *Rhizoctonia solani* Kühn, a bioassay for cotton damping-off pathogens, and total microbial respiration.

2.3. Phyllosphere microorganisms

Leaf samples were removed from iced shipping containers and organized into a single stack. A 1.5 cm diameter cork borer was used to core through the assembled leaves, producing one disc per leaf. Samples of 10 g of the leaf discs thus produced were randomly selected and placed in Tekmar sterile lab bags (Tekmar Co., Cincinnati, OH, USA) with 10 ml 0.1 M potassium phosphate buffer, pH 7.0. The leaf samples were triturated for 1 min with a Tekmar Stomacher Lab Blender Model 80 (Fisher Scientific, Atlanta, GA, USA), following which the liquid portion of each sample was serially diluted and plated with a Spiral Plater Model D (Spiral Systems, Bethesda, MD, USA) on soybean-casein digest agar (Difco Laboratories, Detroit, MI, USA) and nutrient agar (Difco) containing 0.2% amorphous chitin (Rodríguez-

Kábana et al., 1983) for enumeration of actinomycetes and bacteria, and Ohio Experiment Station agar for enumeration of fungi. All plates were incubated at 30°C for 48 h following which bacteria were counted, and for 72 h when fungi and actinomycetes were counted. Populations were expressed as colony forming units (cfu) per gram fresh leaf weight.

2.4. *Rhizosphere microorganisms*

Rhizosphere populations of fungi and bacteria were assessed according to the protocol described by Johnson and Curl (1972). Soil adhering to roots was scraped or brushed from the eight root systems collected from each split plot. This rhizosphere soil was pooled and 0.1 ml of a 1:20 soil:water suspension (thoroughly mixed on a magnetic stirrer) was mixed with Ohio Experiment Station agar for fungi and 0.1 ml of a 1:2000 suspension mixed with Thornton's standardized medium for bacteria (including actinomycetes) in standard petri dishes. Ten replicate plates of each medium were used for each suspension. Oven-dry weights of the quantity of soil plated in the suspensions were determined. After incubation for 8 days at 28°C, colonies enumerated per plate were recorded as cfu per gram soil dry weight.

2.5. *Mycorrhizae*

Lateral and fine roots were removed from cotton taproots and from root-zone soil and were processed according to procedures modified (E.G. O'Neill, personal communication, 1991) from Kormanik and McGraw (1982) for vesicular-arbuscular mycorrhizae (VAM). Roots were placed in Histoprep plastic capsules (Fisher Scientific, Pittsburgh, PA, USA) and the capsules were placed in formalin-acetic acid-alcohol (FAA) fixing solution. The FAA solution was prepared using 113 ml formaldehyde, 302 ml 100% ethanol, 19 ml glacial acetic acid, and 566 ml sterile distilled water. Numbers were randomly assigned to the eight samples from each split plot and were coded on the tissue capsules to prevent bias in VAM assessments.

Roots in the capsules were stored in FAA for approximately 9 months before VAM assessment. Capsules were removed from FAA and rinsed thoroughly with tap water, then placed in heated (90°C) 10% KOH for 30 min to clear the roots, removed, and rinsed thoroughly with tap water until no trace of color could be detected in the rinse. Capsules were placed in 1% HCl for 10 min to acidify the roots and then placed in heated (90°C) lactic acid/acid fuchsin stain (875 ml lactic acid, 63 ml glycerin, 53 ml sterile distilled water, and 10 ml 1% aqueous acid fuchsin solution) for 30 min. The capsules were then removed from the staining solution and placed in a destaining solution (875 ml lactic acid, 63 ml glycerin, and 63 ml sterile distilled water) overnight (12 h). Cotton roots were removed from the capsules, placed in grid-bottomed petri plates, and quantified by direct counting with a dissecting microscope. Each intersection of a cotton root with a petri dish grid line was examined for colonization by VAM fungi. Percent mycorrhizae was determined by dividing the number of root intersections demonstrating VAM colonization by the total number of intersections examined for each sample.

2.6. *Nematodes*

Nematodes were extracted from root-zone soil using the methods described by Rodríguez-Kábana and Pope (1981). Root-zone soil (100 cm³) was spread onto tissue paper on a sieve constructed from 15 cm diameter PVC pipe sections and 1 mm mesh fiberglass screen. The sieve with the soil was placed in a 2.3 l plastic bowl containing 1.3 l sterile water so as to just cover the soil. The soil was incubated at room temperature (25–27°C) for 72 h. The bowl contents were passed through a 250 µm stainless steel sieve (to remove debris) stacked on a 38 µm stainless steel sieve (to trap the nematodes). Nematodes retained were transferred to a counting dish and their numbers determined by direct counting with a dissecting microscope.

2.7. *Soil microarthropods*

Soil cores were extracted for relative populations of Collembola and Acari by a modified version of the Tullgren system as described by Wiggins and Curl (1979). Soil samples in large funnels, with stems positioned over water in a collecting tube, were arranged in series under 40 W light bulbs. The animals migrating in advance of the slowly drying soil (5–7 days) were collected live. Populations were expressed as numbers per kg of air-dried soil. Collembola from soil were also pooled by CO₂ treatment, washed in sterile water with Tween-20 surfactant, triturated in micro-grinders, and the suspensions plated in Ohio medium and in potato dextrose agar with streptomycin for assessment of the principal genera of gut fungi.

2.8. *Rhizoctonia spp.*

Rhizoctonia infestation of root-zone soil was estimated by the stem-trap method of Papavizas and Davey (1959), modified to use soybean leaf-petiole sections. Fresh root-zone soil, which contained cotton root fragments, was adjusted to 60% moisture holding capacity in duplicate deep culture dishes (390 ml capacity) and 40 air-dried, sterilized soybean petiole sections (8 mm length) were buried in the soil in each dish. After 4 days at 28°C, the sections were recovered, serially washed, blotted and plated on the surface of 18% water agar amended with streptomycin sulfate. These were incubated for 20 h at 28°C and examined for identifying morphological features of emerging *Rhizoctonia* hyphae using a compound microscope. Relative infestation was recorded as percentage of stem traps colonized by the pathogen.

2.9. *Cotton bioassay*

A portion of the root-zone soil was assayed for root injury that may be attributed to common damping-off fungi. Soil from each split plot was distributed into ten growth cones (2.5 × 15.5 cm; Ray Leach Container Nursery, Canby, OR, USA) and one cotton seed (DES 119) was sown per cone. Cones were randomized under fluorescent plus incandescent lighting at room temperature (28–30°C) for 14 days

with watering as required. Percentage emergence, shoot length, and post-emergence disease index of washed roots on a 0–5 (healthy–dead) scale were recorded.

2.10. Dehydrogenase assay

Roots and other debris were removed from a portion ($\approx 200 \text{ cm}^3$) of the root-zone soil. The soil was then passed through a 2 mm mesh stainless steel sieve until 10–20 g of sieved soil was collected. Dehydrogenase activity, which is a reliable index of microbial activity in soil (Stevenson, 1959), was determined from modified procedures described by Tabatabai (1982). Sieved soil ($\approx 1 \text{ g}$) for triplicate subsamples from each root-zone soil sample was placed in test tubes ($15 \times 100 \text{ mm}$), covered with 1 ml of 3% aqueous (w/v) 2,3,5-triphenyltetrazolium chloride and stirred with a glass rod. After 96 h incubation (27°C), 10 ml of methanol was added to each test tube and the suspension was vortexed for 30 s. Tubes were then incubated for 1 h to allow suspended soil to settle. The resulting supernatant ($\approx 5 \text{ ml}$) was carefully transferred to clean test tubes using Pasteur pipets. Absorbance was read spectrophotometrically at 485 nm and formazan concentration was calculated using a standard curve produced from known concentrations of triphenyl formazan. One subsample of sieved soil ($\approx 1 \text{ g}$) from each root-zone soil sample was used for determination of soil moisture so that formazan concentrations could be expressed per gram soil dry weight.

2.11. Data analyses

All analyses were performed using the general linear models procedure of the Statistical Analysis System Institute Inc. (SAS Institute Inc., 1982). In all cases, one value per split plot, which was often an average over replicate plants or replicate samples, was used for statistical analyses. Significance in microbial variables between the two CO_2 treatments was tested using the mean square for the block by CO_2 interaction as the error mean square. If the CO_2 by soil moisture interaction was significant, contrast statements were used to determine the significance between the CO_2 treatments within each soil moisture treatment. Differences were considered significant at the $P < 0.10$ level. Values which differed at the $0.10 < P < 0.20$ level were considered trends.

3. Results and discussion

No significant differences were observed between CO_2 or soil water content treatments in phyllosphere bacteria or actinomycete populations at either sampling date; however, there was a trend ($P = 0.17$) for actinomycetes to have higher numbers in the wet soil water treatment at the June sampling. Even though cotton plants growing under elevated CO_2 tended to have higher numbers of leaves and leaf area per plant (S.A. Prior, unpublished, 1991), populations of bacteria and actinomycetes per mm^2 of leaf tissue were not affected by CO_2 level. These data indicate that bacteria and

actinomycetes were able to colonize the additional leaf tissue in numbers comparable with ambient conditions; thus, any changes in leaf structure or nutrient content under FACE did not alter colonization by these microbes. Populations were several orders of magnitude lower for the August compared with the June sampling, which could be a result of insecticides applied between the sampling periods and possibly influenced by a sandstorm which occurred the day before leaf sampling.

Populations of fungi on cotton leaves were affected by CO₂; numbers of *Penicillium* spp. were lower ($P = 0.08$), numbers of *Aspergillus* spp. were unchanged ($P = 0.59$), and numbers of fungi in other genera were higher ($P = 0.07$) under FACE. Reasons for the differential response of the various fungi are not understood, although it is logical that different fungi will respond differently to direct and indirect effects of elevated CO₂. The effects of the August sandstorm on fungal populations and possible interactions with CO₂ concentration are also not known. There was no effect of soil water content on populations of cotton leaf fungi, and no interactions of CO₂ level with soil water treatment were detected for any phyllosphere variable assessed.

There were no significant differences between CO₂ or soil water treatments for microorganisms in rhizosphere soil at either sampling date, except for the total number of fungi which were significantly higher ($P = 0.05$) under wet soil water conditions in the August sampling. As with the phyllosphere, larger root systems of cotton plants growing under elevated CO₂ (Prior et al., 1994) did not influence numbers of rhizosphere microbes. The lack of a significant CO₂ effect on soil microbial populations has also been observed in previous years of FACE work (Rogers et al., 1993; R. Ankumah, personal communication, 1993).

No significant differences between CO₂ treatments were observed for most types of nematodes examined. Similar results were reported by Freckman et al. (1991), who found that the density of nematodes in cores of prairie soil from stands of western wheatgrass and blue grama grass were unaffected by CO₂ concentration in the atmosphere. However, in this study, the number of saprophagous nematodes was significantly higher, and there was a trend for the total number of parasitic nematodes to be lower under FACE (Table 1). The increased number of saprophagous nematodes may account for the lower number of total parasitic nematodes under elevated CO₂ if competition for space or resources occurred between these two types of nematodes. The only significant effect of soil water content occurred for stunt nematodes (*Tylenchorhynchus* spp.), which were higher ($P = 0.02$) under wet soil conditions in August. There was also a trend ($P = 0.15$) for higher numbers of spiral (*Helicotylenchus* spp.) nematodes under the wet soil treatment in the June sampling. A significant interaction between CO₂ and soil water content existed for populations of saprophagous nematodes in June. Numbers of saprophagous nematodes were significantly higher under FACE in the dry soil treatment (214 cm⁻³ soil and 167 100 cm⁻³ soil, for 550 μmol mol⁻¹ and 370 μmol mol⁻¹, respectively; $P = 0.04$), but were not significantly different under wet soil conditions (165 cm⁻³ soil and 182 100 cm⁻³ soil, for 550 μmol mol⁻¹ and 370 μmol mol⁻¹, respectively; $P = 0.39$).

One limitation to the size of a specific nematode population is the amount of substrate available as a food source. The lack of differences between treatments in

Table 1

Population of nematodes in root-zone soil from cotton plants grown under ambient or elevated CO₂ for June and August 1991 samplings

Nematode	CO ₂ concentration ($\mu\text{mol mol}^{-1}$)		<i>P</i> > <i>F</i>
	370	550	
<i>June</i>			
Stunt	11.3 ± 1.2 ^a	9.7 ± 1.2	0.52 ^b
Spiral	3.8 ± 1.0	0.8 ± 0.3	0.32
Root-knot larvae	6.2 ± 3.9	0.5 ± 0.3	0.42
<i>Aphelenchus</i>	9.1 ± 1.0	9.9 ± 1.0	0.27
Total parasitic	30.4 ± 4.0	20.9 ± 1.7	0.17
Saprophagous	174.4 ± 8.1	189.5 ± 7.2	0.08
Dorylamoid	11.4 ± 1.3	11.8 ± 1.3	0.77
Total	216.2 ± 9.2	222.2 ± 8.2	0.61
<i>August</i>			
Stunt	49.1 ± 5.0	54.2 ± 4.1	0.60
Spiral	16.9 ± 7.0	1.0 ± 0.3	0.37
Root-knot larvae	51.9 ± 23.5	3.6 ± 1.8	0.42
Total parasitic	118.0 ± 24.5	58.7 ± 4.5	0.37
Saprophagous	215.7 ± 11.2	272.1 ± 13.4	0.09
Dorylamoid	55.9 ± 3.1	58.6 ± 3.1	0.57
Total	389.6 ± 29.3	389.4 ± 15.0	0.99

^aMean number of nematodes/100 cm³ of root-zone soil ± standard error (*n* = 8).^bProbability of a greater *F* value by chance for the difference between the 370 and 550 $\mu\text{mol mol}^{-1}$ CO₂ treatments.

rhizosphere bacteria would then explain the lack of differences in dorylamoid nematodes, which feed primarily on these bacteria. Also, the additional plant biomass (Prior et al., 1994) and organic carbon (Wood et al., 1994) observed under elevated CO₂ would then account for the higher numbers of saprophagous nematodes found under high CO₂ at both sampling dates. Substrate limitations do not explain the interaction of CO₂ concentration with soil water content on numbers of saprophagous nematodes, as more plant material (Prior et al., 1994) and more organic carbon (Wood et al., 1994) were generally found under elevated CO₂ in wet soil conditions. However, if soil water in the wet treatment was excessive enough to also be limiting to the saprophagous nematode population, this could account for the lack of difference observed under FACE in wet soil and could explain the higher numbers under FACE in dry soil. Changes in numbers of saprophagous nematodes could then explain the interaction of CO₂ with soil water on carbon turnover (Wood et al., 1994) which was unaffected by FACE under wet soil conditions, but was significantly higher under FACE in dry soil.

There were no significant differences between CO₂ or soil water treatments and there was no significant interaction of these test variables on soil microarthropods. However, populations of both Collembola and Acari were extremely low in this clay soil as compared with relatively high numbers common in sandy soil (Wiggins

Table 2

Other microbiological variables measured using roots or root-zone soil from cotton plants grown under ambient or elevated CO₂ for the June and August 1991 samplings

Variable	CO ₂ concentration ($\mu\text{mol mol}^{-1}$)		<i>P</i> > <i>F</i>
	370	550	
<i>June</i>			
Formazan ^a	14.4 ± 0.6 ^b	16.4 ± 0.5	0.08 ^c
Disease rating ^d	1.3 ± 0.1	1.4 ± 0.1	0.62
<i>Rhizoctonia</i> ^e	93.8 ± 1.9	92.5 ± 2.1	0.83
Mycorrhizae ^f	31.3 ± 1.4	35.8 ± 1.6	0.17
<i>August</i>			
Formazan	8.7 ± 0.4	10.4 ± 0.4	0.04
Disease rating	1.6 ± 0.1	1.3 ± 0.1	0.28
<i>Rhizoctonia</i>	58.8 ± 3.9	68.1 ± 3.7	0.17
Mycorrhizae	36.5 ± 1.3	37.6 ± 1.4	0.38

^aQuantity of formazan ($\mu\text{g g}^{-1}$ soil dry weight) from dehydrogenase assay.

^bMean ± standard error (*n* = 8).

^cProbability of a greater *F* value by chance for the difference between the 370 and 550 $\mu\text{mol mol}^{-1}$ CO₂ treatments.

^dAverage disease rating (0–5, healthy to dead) for root systems of cotton plants grown for bioassay of rhizosphere pathogens.

^ePercent of soybean petiole sections colonized by *Rhizoctonia* spp. during incubation in root-zone soil.

^fPercent of cotton root systems colonized by vesicular–arbuscular mycorrhizal fungi.

et al., 1979). Numbers of Collembola tended (*P* = 0.13) to be higher under FACE in August and higher (*P* = 0.14) under wet soil conditions in June, while Acari tended (*P* = 0.15) to be lower under wet soil conditions in August. Microarthropod numbers may reflect differences in rhizosphere fungi, the primary food source for the microarthropods. However, this is not supported by the significantly higher number of total fungi in the wet soil treatment in August, nor by the significantly higher (*P* < 0.01) quantity and quality (i.e. species; data not shown) of Collembola gut fungi in the June sampling. Overall low numbers of microarthropods recovered and high sample to sample variability are the most likely explanations for the lack of differences and for the highly variable trends observed in microarthropods in this study.

Mycorrhizae benefit host plants in several ways, i.e. absorption of nutrients, protection from pathogens; but the exact benefits to plants from differing quantities of mycorrhizae are essentially unknown. Per cent mycorrhizal colonization of cotton roots was similar between the two CO₂ treatments; however, there was a trend for per cent mycorrhizae to be higher under FACE for the June sampling (Table 2). Root systems of FACE plants were larger (Prior et al., 1994) and were as mycorrhizal (slightly more so in June) as plants grown under ambient conditions; therefore, FACE plants should have more mycorrhizae (and hence increased nutrient uptake) on a whole-plant basis. There was a trend for per cent mycorrhizae to be higher in the dry soil water content treatment for both samplings. Excessively high soil moisture can reduce infection by mycorrhizal fungi (Reid and Bowen, 1979) which could

account for the observed trends for cotton roots in the wet soil moisture treatment to be less mycorrhizal.

A trend ($P = 0.17$) for increased *Rhizoctonia* infestation of soil under FACE was observed in August (Table 2). This increase might suggest a potential for increased root disease because of *R. solani* for plants grown under elevated CO_2 , particularly because root length is increased under these conditions (Prior et al., 1994). However, this was not supported by the bioassay disease rating for damping-off pathogens, which indicated no significant effect of CO_2 concentration or soil water content (Table 2). Such conflicting data only emphasize the complexity of interactions of host plants with pathogens or of pathogens with other (possibly biocontrol) microorganisms under conditions of increasing CO_2 .

The quantity of formazan in root-zone soil was significantly higher under FACE at both sampling dates (Table 2), which implies higher microbial activity and thus higher total numbers or activity of soil microorganisms under elevated CO_2 . The increase in numbers of rhizosphere and root-zone microorganisms, both those which increased significantly and those which were numerically (but not statistically) higher, most likely accounted for this increase in microbial activity under FACE. Others have also observed numerical, but not statistical, increases in fungal and bacterial populations in rhizosphere soil from FACE cotton plants (R. Ankumah, personal communication, 1993).

It was expected that additional shifts, either positive or negative, with elevated CO_2 and additional interactions of CO_2 with soil water content would be detected. Even though root growth is often stimulated more by elevated CO_2 than aboveground growth (Wittwer, 1978) it is likely that effects on the rhizosphere and associated microorganisms are more subtle, particularly with an increase of only $\approx 200 \mu\text{mol CO}_2 \text{ mol}^{-1}$. Populations of microorganisms are dynamic and it is possible that, with only two sample periods, microbes were not assessed at optimal times for detecting differences. Also, sample to sample variability was high in most populations assayed, so it is possible that sample sizes were not large enough to detect shifts in populations caused by the treatment variables. High sample variability also accounted for the lack of a CO_2 effect on populations of bacteria and fungi in rhizosphere soil in 1990 FACE research (R. Ankumah, personal communication, 1993).

4. Conclusions

With changes in the morphology and physiology of plants, and their above- and belowground component parts, changes in the number and species of associated microorganisms could occur under elevated CO_2 . Although few significant CO_2 induced changes were observed in phyllosphere and rhizosphere microorganisms in this study, shifts in microbial populations were found which could impact several aspects of plant health and productivity, i.e. turnover of organic matter, nutrient availability, and disease. This vital aspect of plant ecophysiological response to elevated CO_2 deserves further research.

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