ELSEVIER

Agricultural and Forest Meteorology 70 (1994) 87–101

AGRICULTURAL AND FOREST METEOROLOGY

Carbon isotope dynamics of free-air CO_2-enriched cotton and soils

Steven W. Leavitt*[,a], Eldor A. Paul[b], Bruce A. Kimball[c], George R. Hendrey[d], Jack R. Mauney[e], Roy Rauschkolb[f], Hugo Rogers[g], Keith F. Lewin[d], John Nagy[d], Paul J. Pinter, Jr[c], Hyrum B. Johnson[h]

[a]*Laboratory of Tree Ring Research, University of Arizona, Tucson, AZ 85721, USA*
[b]*Department of Crop and Soil Sciences, Michigan State University, East Lansing, MI 48824-1325, USA*
[c]*US Water Conservation Laboratory, USDA, Agricultural Research Service, 4331 East Broadway Road, Phoenix, AZ 85040, USA*
[d]*Department of Applied Sciences, Brookhaven National Laboratory, Upton, NY 11973, USA*
[e]*Western Cotton Research Laboratory, USDA, Agricultural Research Service, 4135 East Broadway Road, Phoenix, AZ 85040, USA*
[f]*Maricopa Agricultural Center, University of Arizona, Maricopa, AZ 85239, USA*
[g]*National Soils Dynamics Laboratory, USDA, Agricultural Research Service, P.O. Box 3439, Samford Ave. and Donahue, Auburn, AL 36831, USA*
[h]*Grassland Soil and Water Research Laboratory, USDA, Agricultural Research Service, 808 Blackland Road, Temple, TX 76502, USA*

Received 25 August 1992; revision accepted 22 November 1993

Abstract

A role for soils as global carbon sink or source under increasing atmospheric CO_2 concentrations has been speculative. Free-air carbon dioxide enrichment (FACE) experiments with cotton, conducted from 1989 to 1991 at the Maricopa Agricultural Center in Arizona, maintained circular plots at 550 μmol mol^{-1} CO_2 with tank CO_2 while adjacent ambient control plots averaged about 370 μmol mol^{-1} CO_2. This provided an exceptional test for entry of carbon into soils because the petrochemically derived tank CO_2 used to enrich the air above the FACE plots was depleted in both radiocarbon (^{14}C content was 0% modern carbon (pmC)) and ^{13}C ($\delta^{13}C \approx -36‰$) relative to background air, thus serving as a potent isotopic tracer. Flask air samples, and plant and soil samples were collected in conjunction with the 1991 experiment. Most of the isotopic analyses on the plants were performed on the holocellulose component. Soil organic carbon was obtained by first removing carbonate with HCl, floating off plant fragments with a NaCl solution, and picking out remaining plant fragments under magnification. The $\delta^{13}C$ of the air above the FACE plots was approximately -15 to $-19‰$, i.e. much more ^{13}C depleted than the background air of approximately $-7.5‰$. The $\delta^{13}C$ values of

* Corresponding author.

Elsevier Science B.V.
SSDI 0168-1923(93)05026-O

plants and soils in the FACE plots were 10–12‰ and 2‰ ^{13}C-depleted, respectively, compared with their control counterparts. The ^{14}C content of the FACE cotton plants was approximately 40 pmC lower than that of the control cotton, but the ^{14}C results from soils were conflicting and therefore not as revealing as the $\delta^{13}C$ of soils. Soil stable-carbon isotope patterns were consistent, and mass balance calculations indicate that about 10% of the present organic carbon content in the FACE soil derived from the 3 year FACE experiment. At a minimum, this is an important quantitative measure of carbon turnover, but the presence of ^{13}C-depleted carbon, even in the recalcitrant 6 N HCl resistant soil organic fraction (average age 2200 years before present (BP)), suggests that at least some portion of this 10% is an actual increase in carbon accumulation. Similar isotopic studies on FACE experiments in different ecosystems could permit more definitive assessment of carbon turnover rates and perhaps provide insight into the extent to which soil organic matter can accommodate the 'missing' carbon in the global carbon cycle.

1. Introduction

Major anthropogenic sources of excess CO_2 to the atmosphere are fossil-fuel burning and land-use changes, especially in the tropics (Post et al., 1990). However, more carbon is being produced from these sources than can be accommodated by both the atmospheric increase and the potential rates of uptake by ocean and biosphere, resulting in a seemingly unbalanced global carbon budget (Post et al., 1990). Soils represent a tremendous pool of carbon (about 1500 Pg) and dominate the carbon in terrestrial ecosystems world-wide (Post et al., 1990). In an enhanced-greenhouse world it is not clear whether soils will exacerbate or mediate the rise in CO_2. Rosenberg (1981) speculated that CO_2 fertilization of plants should yield increased accumulation of soil organic matter. Schlesinger (1990) found that prospects for net soil carbon uptake are poor, especially with regard to the refractory humus component. Bonan (1991), however, found that boreal forest ecosystems are accumulating carbon, most notably in the undecomposed plant materials at the soil surface. In general, the likelihood of enhanced soil carbon accumulation will depend on both carbon input and microbial decomposition. Van Veen et al. (1991) speculated that any heightened microbial decomposition would focus on the increased supply of new, easily decomposable plant matter and exudates, allowing a build-up of older, more resistant soil carbon. Lekkerkerk et al. (1990) found evidence from a short-term study of wheat grown at elevated CO_2 concentrations that the input of easily decomposable root-derived material, preferred by microorganisms as an energy source, increased relative to input from plants under ambient CO_2. Consequently, turnover of more resistant native soil organic matter was reduced and the amount of new organic matter (^{14}C labelled) was also increased under high CO_2.

Carbon isotopic tracers provide an opportunity to resolve questions of carbon loss or gain by soils. In addition to the well-known, traditional radioactive ^{14}C isotopic tracer, there exist stable-carbon isotope tracers (expressed as the $^{13}C/^{12}C$ ratio). All the carbon isotope tracers are effective when the input carbon has a distinctive

isotopic composition relative to the carbon already resident in the soil. Stable-carbon isotope tracers have already been used in addressing carbon turnover changes associated with the shift from C_3 to C_4 plants growing in agricultural settings (Balesdent et al., 1988; Balesdent and Balabane, 1992).

The application of these carbon isotopic tracers to address the question of soil carbon storage related to rising CO_2 concentrations is neatly incorporated in a set of unique field experiments in which CO_2 enrichment is achieved through use of tank CO_2 with distinctive isotopic composition. This exceptional opportunity to study plant–soil interactions in response to elevated CO_2 concentrations arises through free-air CO_2 enrichment (FACE) experiments (Hendrey, 1992; Hendrey et al., 1992). Without confining walls, the FACE approach allows for the culture of crops or other vegetation under conditions as representative of the future 'high-CO_2' world as is currently possible. We sampled air, plants and soils from the FACE and control plots before, during and after a FACE experimental growing season with cotton plants in 1991, in an effort to characterize the system isotopically and determine whether carbon isotopes provide any evidence for enhanced entry of carbon into the soils under high CO_2 levels.

2. Materials and methods

Experiments at the Maricopa Agricultural Center of the University of Arizona (37.07°N, 111.98°W, 358 m elevation) for three consecutive growing seasons, 1989–1991, employed circular plots (23 m diameter in 1989 and 1990; 25 m in 1991) separated by 110 m within fields of cotton (*Gossypium hirsutum* L. cv. 'Deltapine 77') (Lewin et al., 1994; Mauney et al., 1994). The enriched plots were maintained at 550 μmol mol^{-1} CO_2 during daylight hours, using tank CO_2 and a computer-controlled distribution system (Lipfert et al., 1991). Control plots received ambient CO_2 concentrations (about 370 μmol mol^{-1}). Four replicate pairs of control and FACE plots were maintained during each growing season, and the location of three of the FACE plots was the same for all 3 years (Replicate 3 was moved after the first year, but we did not sample it for this study). The soil is mapped as reclaimed Trix clay loam (25–45% sand, 27–40% clay, bulk density 1400–1550 kg m^{-3}) and classified as fine loamy, mixed (calcareous), hyperthermic Typic Torrifluvent (Post et al., 1988). Irrigation was provided by drip tubing installed at 1 m intervals and about 0.20 m below the surface of the bed.

We collected soil samples from depths of 0–0.30 m (the plow layer) and 0.30–0.60 m in a 'bed' (planting area) and a 'furrow' (between rows) position from two pairs of FACE and control plots in April and October 1991 at the beginning and end, respectively, of the experiment's final growing season. In 1990 and 1991, each plot was subdivided, with one half receiving full standard irrigation and the other receiving about 75% and 67% of full irrigation for 1990 and 1991, respectively; samples were taken from the fully irrigated half. Measurements of root distribution (Rogers et al., 1992) indicate that about 60% of the root biomass occurs within the upper 0.30 m.

Uncultivated soil samples at 0–0.30 m and 0.30–0.40 m depths were collected at two sites about 5 km north of the Maricopa Agricultural Center.

Tank CO_2 $\delta^{13}C$ was measured on samples taken with 40 ml evacuated glass vials; once from the field delivery ports (10 April 1991), once from a mixing box (19 July 1991) and once directly from the CO_2 line. Flask air samples from FACE and control plots were taken with 3 l evacuated glass flasks at approximately monthly intervals during the 1991 growing season. Stopcocks were opened and air was admitted and allowed to equilibrate for about 90 s before closing. Cotton plants were collected at the end of the 1991 season for analysis. Plant and soil materials from previous years were obtained from an archived collection (of H.R.).

Carbon dioxide was separated cryogenically from the air samples. Plant samples were either converted to holocellulose via an acid chlorite method (Leavitt and Danzer, 1993), or analyzed as 'whole tissue' after an initial 10% HCl wash pretreatment to eliminate any carbonate dust in the material. This whole-tissue cotton material was sent to the University of Arizona Radiocarbon Laboratory for analysis. Plant samples were combusted to CO_2 for isotopic analysis in a recirculating micro-combustion system. Soil samples were first sieved at 1 mm to remove large plant fragments and pebbles and a subsample of approximately 100 g was acidified with 0.5–1 N HCl to remove the carbonate fraction. Approximately 5 g of these carbonate-free soil samples were then further picked free of recognizable plant and root fragments under a binocular microscope at 20×. Organic carbon content and $\delta^{13}C$ were determined after combustion of 0.10–0.25 g subsamples of these soil samples in quartz tubes at 900°C in the presence of copper oxide, silver foil and copper turnings (Boutton, 1991). Carbon yield was determined manometrically from the CO_2 combustion product, and CO_2 was then analyzed mass-spectrometrically to determine $\delta^{13}C$, where $\delta^{13}C(\text{in ‰ units}) = (({}^{13}C/{}^{12}C_{\text{sample}})/({}^{13}C/{}^{12}C_{\text{PDB standard}}) - 1) \times 1000$. Isotope ratio mass spectrometer precision is approximately ±0.05‰ for repeated analysis of the same CO_2 gas. Seven separate combustions and analyses of subsamples of one soil sample (FACE 1 furrow, collected on 23 October 1991) produced a mean $\delta^{13}C$ of −23.40‰ with respect to the internationally accepted marine carbonate 'PDB' standard and a standard deviation of 0.25‰, and a manometrically determined mean organic carbon content of 0.60% (SD = 0.03%). To resolve further the isotopic characteristics of the organic carbon, about 3 g of picked soil samples were boiled in 6 N HCl for 18 h and the soluble ('supernatant', more active carbon pool) and resistant ('residue', slowly cycling carbon pool) carbon fractions were collected and analyzed as above. The CO_2 remaining after $\delta^{13}C$ analysis of selected soil and soil fractions was retained for tandem accelerator mass spectrometer (TAMS) radiocarbon analysis at the University of Arizona Accelerator Facility. The ^{14}C content was measured as per cent modern carbon (pmC) and converted into age (year before present (BP)), with A.D. 1950 considered 100 pmC and 0 year BP.

Soil carbonates were analyzed by first reacting selected soil subsamples with hydrochloric acid and cryogenically trapping the evolved CO_2. Yields were determined manometrically and the isotopic composition of the CO_2 was measured.

3. Results and discussion

3.1. Air CO_2

Analyses of the tank CO_2 sample from the delivery line gave a very ^{13}C-depleted $\delta^{13}C$ value of approximately −36‰, consistent with its petrochemical origin. The other tank CO_2 samples were −33.5 and −34.7‰, but they were suspect because they may have contained some background air. The overall similarity of gas $\delta^{13}C$ values suggests no major changes in tank CO_2 isotopic composition with time. The air CO_2 collected on six occasions had average $\delta^{13}C$ of -7.5 ± 0.7‰ ($\bar{x} \pm 1$ SD) and -18.2 ± 3.7‰, respectively, for control and FACE samples. These near-instantaneous flask sampling results are consistent with a FACE air CO_2 isotopic composition of −16.8‰ calculated by isotopic mass balance of about 370 μmol mol^{-1} CO_2 in background air at about −7.5‰ mixing with about 180 μmol mol^{-1} CO_2 at −36‰. Isotopic measurements on holocellulose of C_4 sudangrass, planted among the cotton for the purpose of obtaining an integrated isotopic signature of atmospheric carbon dioxide over the growing season, had mean $\delta^{13}C$ of -20.91 ± 0.70‰ and -10.42 ± 0.55‰ ($n = 12$ each), respectively, in FACE and control plots. Theoretically, C_4 plants (such as sudangrass, corn and sugarcane) better represent the isotopic composition of the atmosphere in their plant tissue because of their limited dependence on the ratio of internal to air CO_2 concentrations (C_i/C_a), compared with the considerable dependence on C_i/C_a by C_3 plants (such as trees, cotton and wheat) which can bias the plant tissue isotopic composition away from representative atmospheric values (Marino and McElroy, 1991). The sudangrass FACE–control $\delta^{13}C$ difference of 10.5‰ subtracted from control air of −7.5‰ also gives an average $\delta^{13}C$ value of air in the FACE plots of −18‰. Analysis of different-aged sudangrass plant tissues indicate that the isotopic differences between treatments were nearly constant through the growing season.

We did not obtain ^{14}C measurements on any tank CO_2 or air samples directly, but the tank CO_2 is assumed to have a ^{14}C content of 0 pmC because of its petroleum origin. The 1991 atmospheric background ^{14}C content was approximately 115 pmC (estimated from Loosli and Oeschger, 1989). A mass balance for air above the FACE plots indicates that the air should have a ^{14}C content of about 77 pmC (about 370 μmol mol^{-1} background air CO_2 at 115 pmC mixing with about 180 μmol mol^{-1} tank CO_2 at 0 pmC). The 1991 control cotton plant average ^{14}C activity of 112 ± 1 μmol mol^{-1} is below the expected background air activity and may indicate a small (about 3%) ^{14}C dilution by 'dead' CO_2. The measured control CO_2 concentration of about 370 μmol mol^{-1}, compared with average 1991 background air of about 356 μmol mol^{-1}, also amounts to about 4% excess CO_2. The $\delta^{13}C$ of control air does not confirm a fossil-fuel (^{13}C-depleted) CO_2 input because it is more ^{13}C enriched than the 1991 background air value of about −8.0‰ (estimated from Keeling et al., 1989). If there is a small input of dead CO_2 to the control sites, a slight spillover from the FACE fields would be an obvious possibility, or there may have been wind-carried inputs from metropolitan centers such as Phoenix, 40 km north.

3.2. Plant composition

The $\delta^{13}C$ composition of the cotton plants (Table 1) reflects the differences in control and FACE atmospheres. Plant material is normally ^{13}C depleted relative to its respective air $\delta^{13}C$ because of selectivity for ^{12}C by the carbon-fixing enzyme, RuBP carboxylase. Regardless of plant tissue/organ, or whether holocellulose or whole tissue was analyzed, the isotopic composition of plants in the FACE plots was typically 10–12‰ more ^{13}C depleted (more negative) than that of the control plants. This isotopic difference persists for both replicate plot pairs and indicates apparent consistency of treatment. There does not appear to be any consistent order of isotopic composition among the various plant organs. Plant material from the first 2 years of the experiment also shows control vs FACE differences similar to those for the 1991 season (Table 1), and indicates fairly uniform conditions from season to season. Analysis of cotton bolls from three distant cotton fields in the surrounding area yielded values of −25.7, −25.9 and −25.9‰. These values are

Table 1
$\delta^{13}C$ (in ‰) of cotton plant matter in two control and FACE plots

	Replicate 1 wet		Replicate 2 wet	
	Control	FACE	Control	FACE
Oct. 1991[a]				
Leaves (hc)[b]	−24.62 (0.37)	−36.22 (0.83)	−25.34 (0.45)	−36.95 (0.32)
Roots (hc)	−25.30 (0.21)	−37.20 (0.88)	−25.35 (0.54)	−37.70 (0.45)
Stems (hc)	−25.50 (0.64)	−37.11 (0.75)	−25.4	−36.56 (0.69)
Cotton (wt)[c]	−26.28 (1.23)	−38.18 (1.60)	−25.8	−38.2
Leaves (wt)	−28.0	−38.9	−27.7	−39.7
Roots (wt)	−25.6	−36.8	−25.7	−38.0
1990[d]				
Leaves (hc)	−26.3	−37.3	−25.8	−35.0
Leaves (wt)	−28.2	−40.4	−28.6	−38.4
Taproot (hc)	−25.7	−38.1	−26.0	−37.2
Fine roots (hc) 0–15 cm	−26.0	−37.9	—	−33.7
Fine roots (hc) 30–45 cm	−26.2	−38.3	−26.8	−35.2
1989[d]				
Leaves (hc)	−25.2	−35.6	−25.3	−35.0
Taproot (hc)	−24.8	−36.6	−26.7	−35.5
Overall averages	−26.0 ± 1.1	−37.6 ± 1.2	−26.2 ± 1.1	−36.7 ± 1.7
Overall averages of both plots, all years and all plant material	Control: −26.1 ± 1.1	FACE: −37.1 ± 1.5		

[a] Where standard deviation appears in parentheses, the two collected cotton plants were analyzed separately.
[b] hc, Holocellulose.
[c] wt, Whole tissue.
[d] Two to four cotton plants were collected in September and pooled during root biomass study (Rogers et al., 1992).

consistent with cotton values in the control plots and suggest that the control plots are not experiencing any significant contamination from CO_2 of the neighboring FACE plots. The whole-tissue analyses were ^{13}C depleted relative to their holocellulose counterparts because of the presence of isotopically light, non-cellulosic compounds, e.g. lignin and lipids (Deines, 1980).

We systematically tested for $\delta^{13}C$ variability within the circular FACE plots by analyzing cotton bolls sampled at different distances from the center at the end of the growing season in both the wet and dry halves of the FACE 1 and FACE 2 plots (Fig. 1). The results indicate there are some systematic differences within the FACE plots; namely, there is a general progression from a more ^{13}C-depleted inner 7 m to less depletion toward the outside. If the mean value of −36.7‰ corresponds to the average of 550 μmol mol^{-1}, then the 1.2‰ standard deviation equates to ±33 μmol mol^{-1} or ±6%, in good agreement with 1991 field CO_2 control estimates that for about 93% of the time the amount CO_2 was controlled within ±10% (Nagy et al., 1994). Alternatively, other environmental factors, such as light and soil moisture, can influence C_i/C_a of C_3 plants (Farquhar et al., 1982) and may be contributing to the apparent radial cotton $\delta^{13}C$ trend in the plots.

The radiocarbon content of cotton plants is summarized in Table 2. Material which had been stored from the 1990 growing season was analyzed along with material collected in October 1991. The average radiocarbon content of plants in the control treatments was 112.7 ± 1.8 pmC, close to that expected for background air, whereas the FACE plants were clearly depleted in radiocarbon with an average of 72.8 ± 3.4 pmC. This latter value is not significantly different from the approximate 77 pmC expected on the basis of mass balance considerations above.

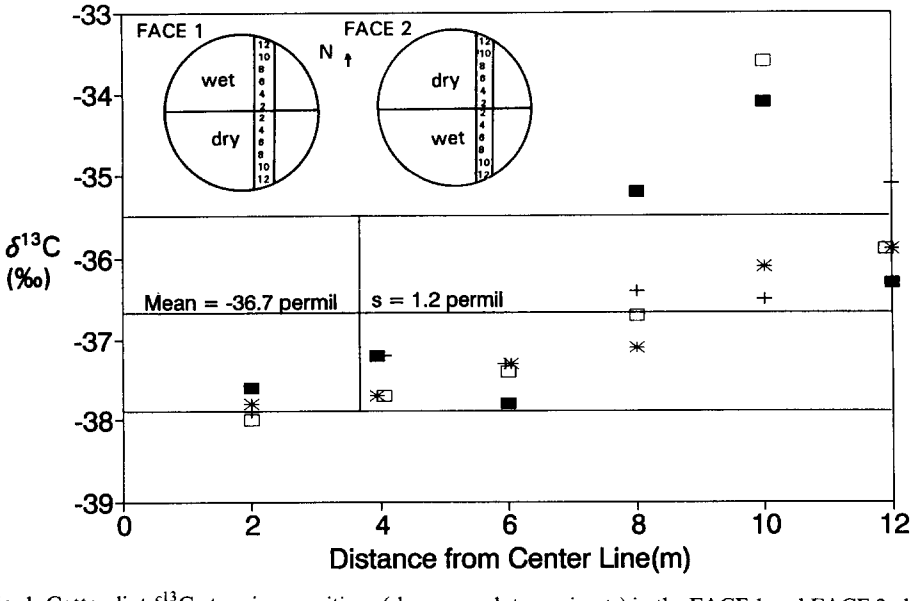

Fig. 1. Cotton lint $\delta^{13}C$ at various positions (shown on plot map insets) in the FACE 1 and FACE 2 plots. FACE 1 dry (■), FACE 1 wet (+), FACE 2 dry (*), FACE 2 wet (□).

Table 2
Radiocarbon content (per cent modern carbon, pmC) of control and FACE cotton plants

Plot	Organ	Experiment year[a]	Radiocarbon content (pmC)
Control 1 wet	Leaves	1990	112.6 ± 0.5
FACE 1 wet	Leaves	1990	70.8 ± 0.4
Control 2 wet	Leaves	1990	116.0 ± 0.5
FACE 2 wet	Leaves	1990	77.5 ± 0.3
Control 1 wet	Leaves	1991	112.1 ± 0.4
FACE 1 wet	Leaves	1991	73.7 ± 0.4
Control 2 wet	Leaves	1991	111.3 ± 0.4
FACE 2 wet	Leaves	1991	71.0 ± 0.3
Control 1 wet	Roots	1991	113.0 ± 0.4
FACE 1 wet	Roots	1991	75.5 ± 0.4
Control 2 wet	Roots	1991	111.1 ± 0.4
FACE 2 wet	Roots	1991	68.2 ± 0.4

[a] The 1990 samples were collected in September; 1991 samples were collected in October.

3.3. Soils

One of the major soil carbon pools in arid-region soils is carbonate carbon. We have measured an average of approximately 2% $CaCO_3$ in these cotton field soils (about 0.25% carbonate carbon). It appears that $\delta^{13}C$ of the FACE carbonates is 0.1–0.2‰ more ^{13}C depleted than in the control soils (Table 3), but these differences are minor and largely within the limit of our experimental error. Likewise there is no real $\delta^{13}C$ difference between the April and October soil samples. Further, the ^{14}C ages of the carbonate carbon of control and FACE samples of this inorganic carbon from both 16 April and 23 October are between 2100 and 2700 years BP (2420 ± 170 years), and thus this soil carbon fraction appears to be very inactive.

The organic soil carbon has acquired the FACE stable-carbon isotopic signal, with the soils in the FACE plots about 2‰ ^{13}C depleted relative to those in the control plots (Table 4). This pattern exists for both bed and furrow sites, and the isotopic differences were reproduced in both of the control–FACE pairs. This $\delta^{13}C$ difference is also seen in successive years of the experiment, with average 1991 FACE 1 and

Table 3
Carbonate $\delta^{13}C$ and ^{14}C results from 0–0.30 m 'bed' position soil samples

Plot	Date of sample	$\delta^{13}C$ (‰)	^{14}C activity (pmC)	^{14}C age (years BP)
Control 1 wet	16 April 1991	−4.6	74.8 ± 0.5	2335 ± 60
FACE 1 wet	16 April 1991	−4.9	77.0 ± 0.5	2100 ± 55
Control 2 wet	16 April 1991	−4.7	74.4 ± 0.5	2380 ± 60
FACE 2 wet	16 April 1991	−4.8	73.6 ± 0.5	2460 ± 55
Control 1 wet	23 October 1991	−4.6	74.0 ± 0.5	2417 ± 58
FACE 1 wet	23 October 1991	−5.0	72.9 ± 0.7	2544 ± 76
Control 2 wet	23 October 1991	−4.5	71.7 ± 0.6	2672 ± 66
FACE 2 wet	23 October 1991	−4.7	73.6 ± 1.0	2468 ± 105

control 1 soil $\delta^{13}C$ values of −24.30‰ and −22.17‰, respectively, compared with 1990 values of −23.20‰ and −22.01‰, respectively, and 1989 values of −23.18‰ and −22.39‰, respectively. Furthermore, the signal was seen in both the 6 N HCl supernatant and 6 N HCl residue fractions of the picked samples, with the residue being the more ^{13}C-depleted fraction. The $\delta^{13}C$ values in the control 6 N HCl residue fraction of −22.2 to −23.4‰ could represent inputs from cultivation of C_3 plants such as cotton over the past 40 years or so, but the low ^{14}C contents of this fraction (see below) indicate ages of 1500–3500 years BP and suggest that the native plants which preceded cultivation at this site were also dominantly ^{13}C-depleted C_3 plants. This is supported by the uncultivated soil samples whose organic carbon at 0–0.30 m depth averaged −21.4‰ compared with an average of −23.3‰ at 0.30–0.40 m depth. The soil samples taken just before the beginning of the 1991 growing season also show the same pattern, with the exception of an unusually ^{13}C-depleted value (−23.23‰) for the control 2 furrow location.

Additional $\delta^{13}C$ analyses were conducted on soils from the 0.30–0.60 m soil depth

Table 4
$\delta^{13}C$ (in ‰) of soil organic carbon from the 0–0.30 m depth

	Control		FACE	
	Bed	Furrow	Bed	Furrow
Replicate 1 wet				
April 1991				
Picked	−22.74 (0.88)[3][a]	—	−23.85 (0.31)[3]	—
Oct. 1991				
Picked	−22.54 (0.08)[2][b]	−21.79 (0.25)[2]	−25.19 (0.25)[2]	−23.40 (0.25)[7]
HCl supernatant	−21.35 [1]	−21.13 [1]	−22.75 [1]	−22.41 (0.35)[3]
HCl residue	−22.94 [1]	−22.24 (0.10)[2]	−25.30 [1]	−24.24 [1]
Replicate 2 wet				
April 1991				
Picked	−21.82 (0.37)[3]	−23.23(0.22)[2]	−23.43 (1.03)[4]	−23.23 [1]
Oct. 1991				
Picked	−22.73 [1]	−21.88 (0.55)[2]	−23.37 [1]	−24.64 (0.09)[2]
HCl supernatant	−20.87 [1]	−21.04 [1]	−22.43 [1]	−22.53 (0.35)[2]
HCl residue	−23.41 (0.28)[1]	−22.77 [1]	−24.22 [1]	−24.78 (0.44)[2]

		Control	FACE	Percentage of Organic C[b]	Δ
Overall averages of both plots					
April 1991	Picked	−22.60‰(0.72‰)	−23.50 (0.32)	100	0.90‰ (ns)[c]
October 1991	Picked	−22.24 (0.47)	−24.15 (0.91)	100	1.91 ($P < 0.05$)
	Supernatant	−21.09 (0.20)	−22.53 (0.16)	36	1.44 ($P < 0.01$)
	Residue	−22.84 (0.48)	−24.64 (0.51)	64	1.80 ($P < 0.01$)

[a] Standard deviation given in parentheses; no. of combustions of separate subsamples in brackets.
[b] Distribution of organic carbon among fractions.
[c] t-Test significance.

(Table 5), and on various size fractions of the soils (Table 6). The deeper 0.30–0.60 m soils do not appear to show any isotopic signal related to the enhanced CO_2 levels above the FACE plots; the FACE soils are not significantly ^{13}C-depleted compared with the control soils. This table also demonstrates the importance of multiple analyses in detecting real isotopic difference, in that several picked soil $\delta^{13}C$ values do not fall within the range of the respective supernatant and residue values, as is required by mass balance. The size fractions were obtained by sieving control and FACE 1 0–0.30 m soil samples (carbonates were not removed beforehand, and plant fragments were removed on a 2 mm sieve but were not floated or picked out), and show very generally that the larger fractions of the FACE soils are isotopically much lighter than the fine fraction; this is probably a consequence of the input of fresh plant materials (field stubble and root fragments). The control soil size fraction $\delta^{13}C$ patterns are much more variable. It is important to note that the $\delta^{13}C$ in Table 6 are more ^{13}C-depleted than those in Table 4 as a consequence of the presence of more plant fragments, which carry a much more ^{13}C-depleted signal than the Table 4 soils, from which plant fragments have been floated and picked. Fine carbonates may also contribute to the ^{13}C enrichment with depth.

The overall 0–0.30 m averages in Table 4 include the two replicate plots and the bed/furrow positions as additional cases, and are the key to further quantitative analysis. The $\delta^{13}C$ of October 1991 picked soil samples and soil fractions indicate that control and FACE results were significantly different. The April 1991 control and FACE picked soil samples were not significantly different, but there are fewer cases. It is possible to calculate the fraction of FACE carbon input required to change the $\delta^{13}C$ of the organic carbon content 1.4–1.9‰, as seen in the difference between control and

Table 5
$\delta^{13}C$ (in ‰) of soil organic carbon from the 0.30–0.60 m depth

	Control		FACE	
	Bed	Furrow	Bed	Furrow
Replicate 1 wet				
Oct. 1991				
Picked	−21.6 [1][a]	−19.2 [1]	−21.8 [1]	−19.7 [1]
HCl supernatant	−21.1 [1]	−21.6 [1]	−22.8 [1]	−21.9 [1]
HCl residue	−21.7 [1]	−22.8 [1]	−22.1 [1]	−21.4 [1]
Replicate 2 wet				
Oct. 1991				
Picked	−19.2 [1]	−21.0 [1]	−21.9 [1]	−21.8 [1]
HCl supernatant	−17.4 [1]	−21.9 [1]	−22.5 [1]	−20.3 [1]
HCl residue	−23.2 [1]	−22.7 [1]	−23.5 [1]	−22.9 [1]
Overall average				
Picked	−20.25 ± 1.24		−21.30 ± 1.07	
Supernatant	−20.50 ± 2.09		−21.88 ± 1.11	
Residue	−22.60 ± 0.64		−22.48 ± 0.92	

[a] Number of combustions of separate subsamples given in brackets.

Table 6
$\delta^{13}C$ (in ‰) for various size fractions of Replicate 1 soils collected in October 1991

Size fraction (μm)	0–0.30 m		0.30–0.60 m	
	Control	FACE	Control	FACE
1000–2000	-25.9 ± 0.4[a]	-33.1 ± 2.7	-23.5	-36.3 ± 3.0
500–1000	-24.2 ± 0.7	-33.9 ± 0.3	-26.7 ± 3.2	-38.5 ± 3.2
250–500	-25.2 ± 0.0	-33.3 ± 2.3	-25.4 ± 0.1	-32.3 ± 0.3
125–250	-24.7 ± 0.7	-31.7	-23.3 ± 1.5	-28.1
53–125	-23.8 ± 2.5	-30.7 ± 1.2	-25.1 ± 2.2	-31.3 ± 5.4
< 53	-24.6 ± 1.8	-30.3 ± 2.3	-21.5 ± 1.5	-25.5 ± 2.6

[a] Standard deviations are from multiple analysis of sample.

FACE plots, using isotopic mass balance methods (Balesdent et al., 1988) and the following equation:

$$\delta^{13}C_{\text{FACE soil}} = f_{\text{input}}(\delta^{13}C_{\text{input}}) + f_{\text{soil original}}(\delta^{13}C_{\text{soil original}})$$

If organic matter from the enriched cotton plants was the only new carbon potentially added to the soil, then $f_{\text{soil original}}$ (the fraction of carbon in the current organic pool from 'old' carbon) is equal to $1 - f_{\text{input}}$ (where f_{input} is the fraction of new FACE carbon in the current organic pool), and the percentage of 'new' carbon in the pool can be calculated after making two other assumptions. The first is that the $\delta^{13}C$ of the soil in the control plots can be used as a value for $\delta^{13}C_{\text{soil original}}$ of the FACE plots, and the second is that we can use FACE plot cotton plant isotopic values as representative of $\delta^{13}C_{\text{input}}$. For our calculations, we used the average $\delta^{13}C$ of whole tissue (roots and leaves) of 1991 FACE Plots 1 and 2 for $\delta^{13}C_{\text{input}}$ (-38.35 ± 1.24‰). Solving the above equation for f_{input}, we obtain 12% using the October 1991 picked soil $\delta^{13}C$, 8% using the supernatant $\delta^{13}C$, and 12% with the residue $\delta^{13}C$ values. Calculating with the pre-1991 FACE season picked soil sample, f_{input} is 6%.

Does this fraction of fresh carbon in the organic carbon pools represent a net increase in carbon, or could the size of the carbon pool be unchanged (or even smaller), with new carbon added balanced by loss of old carbon? Even if the latter were the case, this approximate value of 10% would be a major quantitative result in itself as a direct measure of soil organic carbon turnover over the course of the experiment. Organic carbon content on its own was originally reckoned to be not sufficiently sensitive to resolve the question which prompted this isotopic study. In fact, average organic carbon content of our October 1991 picked control soil samples was 0.56 ± 0.12% compared with 0.64 ± 0.10% for the FACE soils. This suggests there is more carbon in the FACE plots, but the difference is not statistically significant. Other independent measures of soil organic carbon content are also conflicting. The work of Wood et al. (1994) on another set of soils collected from the 1991 FACE experiment indicates that the carbon content of 0.10–0.20 m FACE soils is significantly enriched in carbon relative to the control soils. Separate measurements by R. Rauschkolb and H.Y. Cho (unpublished data, 1992) suggest that the organic carbon

content of 0–0.30 m FACE soils in furrow positions was significantly greater than that of control soils, but for the bed position there was no significant difference, although the control soils actually had greater carbon on average.

In principle, ^{14}C measurements on the soil organic carbon can be used for verification in a mass balance identical to that described above. The organic carbon ^{14}C data from the soils collected in April 1991 and October 1991, before and after the cotton growing season, respectively, are summarized in Table 7, but they possess some inconsistencies which do not permit verification of the $\delta^{13}C$ results. For example, the April FACE 1, 6 N HCl residue fraction is suspect because it has a higher ^{14}C content than any of the control residues, but the input of 'dead' CO_2 into the FACE plots should have produced lower ^{14}C contents. However, the pattern of picked-soil ^{14}C activities, intermediate between residue and supernatant fractions, is as expected. It is noteworthy that the residue fraction of all control soils is greater than 1500 years old (2570 ± 810 years BP), yet a substantial FACE $\delta^{13}C$ isotope signal has apparently made its way into the organic carbon of this recalcitrant fraction in only 3 years.

We can take the ^{14}C content of the FACE and control picked soils and perform an isotope mass balance as with the stable-carbon isotopes:

$$^{14}C_{\text{FACE soil}} = f_{\text{input}}(^{14}C_{\text{input}}) + f_{\text{soil original}}(^{14}C_{\text{soil original}})$$

Table 7
^{14}C Content of soil organic carbon, as determined by accelerator (TAMS)

Plot	Treatment	15 April 1991		23 October 1991	
		^{14}C Activity[a]	^{14}C Age[b]	^{14}C Activity	^{14}C Age
Control 1 wet	Picked	102.1 ± 0.7	—	91.6 ± 1.1[c]	703 ± 124
Control 1 wet	6 N HCl supernatant	107.7 ± 0.8	—	97.0 ± 0.7[c]	243 ± 60
Control 1 wet	6 N HCl residue	64.5 ± 0.6	3520 ± 70	73.8 ± 0.8[c]	2436 ± 84
FACE 1 wet	Picked	99.5 ± 0.7	40 ± 60	87.6 ± 1.0[c]	1067 ± 95
FACE 1 wet	6 N HCl supernatant	—	—	96.9 ± 0.7[c]	257 ± 55
FACE 1 wet	6 N HCl residue	94.6 ± 0.7	445 ± 55	82.8 ± 0.8[c]	1516 ± 79
Control 2 wet	Picked	100.8 ± 0.6	< 160	87.0 ± 0.8	1114 ± 75
Control 2 wet	6 N HCl supernatant	—	—	95.8 ± 0.7	342 ± 61
Control 2 wet	6 N HCl residue	82.2 ± 0.6	1570 ± 55	70.8 ± 0.6	2770 ± 67
FACE 2 wet	Picked	85.6 ± 0.6	1245 ± 60	88.3 ± 0.8	1000 ± 73
FACE 2 wet	6 N HCl supernatant	93.3 ± 0.7	558 ± 57	103.2 ± 0.8	—
FACE 2 wet	6 N HCl residue	75.9 ± 0.6	2220 ± 60	66.9 ± 0.5	3234 ± 65

[a] Per cent modern carbon (pmC).
[b] Years BP — when activity exceeds 101 pmC, age is not calculated because it is equivalent to a future date.
[c] Samples from furrow locations; all others were from bed locations.

If (a), $f_{\text{soil original}} = 1 - f_{\text{input}}$, (b), the ^{14}C content of the control soils is representative of the $^{14}C_{\text{soil original}}$ of the FACE plots and (c), the FACE plot cotton ^{14}C values represent $^{14}C_{\text{input}}$, then the FACE inputs can be calculated. For example, with October control and FACE picked average $\delta^{13}C$ values for $^{14}C_{\text{FACE soil}}$ of 88.0 pmC and $^{14}C_{\text{soil original}}$ of 89.1 pmC, and $^{14}C_{\text{input}}$ of 74.6 pmC (1991 leaves and

roots), the f_{input} is equal to 9%, i.e. the same as that calculated from the $\delta^{13}C$ mass balance. However, the real problem with the ^{14}C data is embodied in the residue and supernatant fractions in which the average October control ^{14}C activity is actually less than that of the corresponding FACE soils and the mass balance cannot be calculated. Because of such inconsistencies with ^{14}C content of the soil organic carbon fractions, the ^{14}C mass balance does not add meaningful results.

4. Conclusions

The use of stable-carbon isotopes in plants and soils is especially effective in evaluating the uniformity of experimental treatment and inferring the proportion of 'new' carbon which resulted from FACE treatment over a 3 year period. Similar averaged isotopic results from year to year suggest that treatments have been uniform over the 3 years of experiment, and plant $\delta^{13}C$ differences within FACE plots suggest that CO_2 was held at 550 μmol mol^{-1} $\pm$6% although some of the cotton $\delta^{13}C$ variability could be caused by physiological effects resulting from other environmental differences. The cotton plants in the FACE fields were ^{13}C and ^{14}C depleted relative to those in the control plots, and these tracers have made their way into the shallow 0–0.30 m soil depth, producing a measurable difference in $\delta^{13}C$ of FACE and control soils regardless of bed or furrow field position. This isotopic signal even occurs in the recalcitrant 6 N HCl residue fraction, indicating a substantial contribution from FACE organic matter to the soils. The ^{14}C content of soil organic carbon presents a much less consistent picture and appears less effective than the stable-carbon isotopes in this type of analysis.

The elevated CO_2 levels have left their isotopic mark on the organic carbon pools in the FACE soil. At a minimum, the results indicate that about 10% of the soil carbon was replaced with 'fresh' carbon in 3 years, including the more recalcitrant fractions. The results also provide some indication that the soils may be acting as an enhanced carbon sink under high ambient CO_2. Application of these techniques to CO_2 enrichment studies over several ecosystems may permit a quantitative global assessment of such carbon storage in soil organic matter.

Acknowledgments

We thank the US Department of Agriculture, Agricultural Research Service, and US Department of Energy, Carbon Dioxide Research Program of the Environmental Sciences Division, for supporting the FACE experiments, and the former for supporting this isotopic research project under specific cooperative agreement 58-91H2-0-301 with the University of Arizona. D. Akin kindly provided sudangrass material. D. Harris, M. Farrell, K. DeWitte, M. Kavoosi, P. van de Water, E. Markus, T. Prezelski, S. Danzer and S. Prior all helped with soil and plant preparation. $\delta^{13}C$ analyses of CO_2 were made at the Laboratory of Isotope Geochemistry, and

accelerator radiocarbon dating was carried out at the National Accelerator Facility, both at the University of Arizona.

References

Balesdent, J. and Balabane, M., 1992. Maize root-derived soil organic carbon estimated by natural ^{13}C abundance. Soil Biol. Biochem., 24: 97–101.

Balesdent, J., Wagner, G.H. and Mariotti, A., 1988. Soil organic matter turnover in long-term field experiments as revealed by carbon-13 natural abundance. Soil Sci. Soc. Am. J., 52: 118–124.

Bonan, G.B., 1991. Seasonal and annual carbon fluxes in a boreal forest. J. Geophys. Res., 96: 17329–17338.

Boutton, T.W., 1991. Stable carbon isotope ratios of natural materials: I. Sample preparation and mass spectrometric analysis. In: D.C. Coleman and B. Fry (Editors), Carbon Isotope Techniques. Academic Press, New York, pp. 155–171.

Deines, P., 1980. The isotopic composition of reduced organic carbon. In: P. Fritz and J.Ch. Fontes (Editors), Handbook of Environmental Isotope Geochemistry, Vol. 1. Elsevier, New York, pp. 329–405.

Farquhar, G.D., O'Leary, M. and Berry, J.A., 1982. On the relationship between carbon isotope discrimination and intercellular carbon dioxide concentration in leaves. Aust. J. Plant Physiol., 9: 121–137.

Hendrey, G.R. (Editor), 1992. FACE: Free-air CO_2 enrichment for plant research in the field. Crit. Rev. Plant Sci., 11(2,3), 308 pp.

Hendrey, G.R., Lewin, K.F. and Nagy, J., 1992. Free-air carbon dioxide enrichment: developments, progress, results. Vegetatio, 104–105: 1–16.

Keeling, C.D., Bacastow, R.B., Carter, A.F., Piper, S.C., Whorf, T.P., Heimann, M., Mook, W.G. and Roeloffzen, H., 1989. A three-dimensional model of atmospheric CO_2 transport based on observed winds: 1. Analysis of observational data. Geophys. Monogr. Am. Geophys. Union, 55: 165–236.

Leavitt, S.W. and Danzer, S.R., 1993. Method for batch processing small wood samples to holocellulose for stable-carbon isotope analysis. Anal. Chem., 65: 87–89.

Lekkerkerk, L.J.A., van de Geijn, S.C. and van Veen, J.A., 1990. Effects of elevated atmospheric CO_2 levels in the carbon economy of a soil planted with wheat. In: A.F. Bouwman (Editor), Soils and the Greenhouse Effect. Wiley, New York, pp. 423–429.

Lewin, K.F., Hendrey, G.R., Nagy, J. and LaMorte, R.L., 1994. Design and application of a free-air carbon dioxide enrichment facility. Agric. For. Meteorol., 70: 15–29.

Lipfert, F., Alexander, Y., Lewin, K., Hendrey, G. and Nagy, J., 1991. A turbulence-driven air fumigation facility for studying air pollution effects on vegetation. In: Preprint Volume of 7th Symp. on Meteorol. Obs. and Instrument. and Spec. Sessions on Laser Atmos. Studies. Am. Meteorol. Soc., New Orleans, LA, 14–18 January. American Meteorological Society, Boston, MA, pp. J199–J202.

Loosli, H.H. and Oeschger H., 1989. ^{14}C in the environment of Swiss nuclear installations. Radiocarbon, 31: 747–753.

Marino, B.D. and McElroy, M.B., 1991. Isotopic composition of atmospheric CO_2 inferred from carbon in C_4 plant cellulose. Nature, 349: 127–131.

Mauney, J.R., Kimball, B.A., Pinter, Jr, P.J., LaMorte, R.L., Lewin, K.F., Nagy, J. and Hendrey, G.R., 1994. Growth and yield of cotton in response to a free-air carbon dioxide enrichment (FACE) environment. Agric. For. Meteorol., 70: 49–67.

Nagy, J., Lewin, K.F., Hendrey, G.R., Hassinger, E. and LaMorte, R., 1994. FACE facility CO_2 concentration control and CO_2 use in 1990 and 1991. Agric. For. Meteorol., 70: 31–48.

Post, D.F., Mack, C., Camp, P.D. and Suliman, A.S., 1988. Mapping and characterization of the soils on the University of Arizona Maricopa Agricultural Center. In: Proc. Hydrology and Water Resources in the Southwest, 16 April 1988, Tucson, AZ. Arizona–Nevada Acad. Sci. Proc., 18: 49–59.

Post, W.M., Peng, T.H., Emanuel, W.R., King, A.H., Dale, V.H. and DeAngelis, D.L., 1990. The global carbon cycle. Am. Sci., 78: 310–326.

Rogers, Jr, H.H., Prior, S.A. and O'Neill, E.G., 1992. Cotton root and rhizosphere response to free-air CO_2 enrichment. Crit. Rev. Plant Sci., 11(2,3): 251–263.

Rosenberg, N.J., 1981. The increasing CO_2 concentration in the atmosphere and its implication on agricultural productivity. I. Effects on photosynthesis, transpiration and water use efficiency. Clim. Change, 3: 265–279.

Schlesinger, W.H., 1990. Evidence from chronosequence studies for a low carbon-storage potential of soils. Nature, 348: 232–234.

Van Veen, J.A., Liljeroth, E. and Lekkerkerk, L.J.A., 1991. Carbon fluxes in plant–soil systems at elevated atmospheric CO_2 levels. Ecol. Appl., 1: 175–181.

Wood, C.W., Torbert, H.A., Rogers, H.H., Runion, G.B. and Prior, S.A., 1994. Free-air CO_2 enrichment effects on soil carbon and nitrogen. Agric. For. Meteorol., 70: 103–116.