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Carbon isotopes and carbon turnover in cotton and wheat FACE experiments

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Abstract

The Maricopa cotton and wheat FACE (free-air CO_2 enrichment) experiments offer propitious opportunity to quantify carbon turnover. The commercial CO_2 ($\delta\ ^{13}C \approx -37$ ‰) used to elevate CO_2 concentration in field plots provided a strongly ^{13}C-depleted tracer. Soil CO_2 and $\delta^{13}C$ of soil organic carbon (SOC) in CO_2 -enriched and Control plots were measured between the final cotton FACE project (October 1991) and the end of the second wheat experiment (June 1994). The initial ^{13}C-depletion in SOC of cotton FACE plots (measured by the difference in $\delta^{13}C$ between FACE and Control plots) persisted at the same level (1.9 ‰) 1.5 years after the experiment ended. A similar depletion was observed in soil CO_2 evolved in the same plots, indicating ongoing decomposition of the new SOC. The SOC $\delta^{13}C$ of wheat plots before and after two growing seasons showed increasing ^{13}C-depletion in FACE relative to Control. Isotopic mass balance was consistent with 5–6% new carbon input from the two wheat crops. This is lower than the 12–13% calculated for FACE cotton and perhaps a consequence of the larger root system of cotton or the 3-year duration of the cotton experiments versus 2 years for the wheat.

Introduction

Stable-C isotope measurements in soil have been useful for investigating soil C transformations in systems where the replacement of C_4 plants by C_3 or vice versa provides a distinctive isotopic label for new soil organic C (SOC) (Balesdent et al., 1987, 1988, 1992; Townsend et al., 1995; Van Kessel et al., 1994). Free-air CO_2 enrichment (FACE) experiments also offer exceptional opportunities to characterize C dynamics in the atmosphere-plant-soil system when the commercial CO_2 gas used to raise concentrations is isotopically distinct from background air. FACE experiments were conducted at the University of Arizona's Maricopa Agricutural Center with cotton (*Gossypium hirsutum* L. cv. Deltapine) for three growing seasons (1989-1991) (Dugas and Pinter,1993; Hendrey, 1992) and with wheat (*Triticum aestivum* L. cv. Yecora Rojo) for two growing seasons (1992-93 and 1993-94) (Pinter et al., 1995) by elevating CO_2 concentration to 550 μmol mol^{-1} in enriched plots (FACE plots) with tank CO_2, compared to background (Control) plots at 350–370 μmol mol^{-1} (Table 1).

Experiments employed four FACE and Control replicate plot pairs embedded within a large farm field, each circular plot having a diameter of ca. 23 meters. Carbon dioxide was added through a series of vertical pipes from a circular plenum that bordered each plot. Computer-controlled opening of ports on the pipes maintained CO_2 levels above FACE plots at 550 μmol mol^{-1}. Soil moisture and nutrient levels were maintained by regular irrigation as needed and fertilization.

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Table 1. Plot history of above-plot CO_2 concentration level (amb=ambient, enrich=enriched), planted crop (cotton or wheat), and sampling schedule for isotopic analysis of soil-air CO_2 and SOC from 1989–1994)

	Year, Growing season and treatment					
	1989 summer	1990 summer	1991 summer	1991–92* winter/spr	1992–93 winter/spr	1993–94 winter/spr
Cotton plots						
FACE	Enrich Cotton	Enrich Cotton	Enrich[a] Cotton	Amb[a,b] Wheat	Amb[a] Wheat	Amb Wheat
Control	Amb Cotton	Amb Cotton	Amb[a] Cotton	Amb[a,b] Wheat	Amb[b] Wheat	Amb Wheat
Wheat plots						
FACE	Amb Cotton	Amb Cotton	Amb Cotton	Amb[c] Wheat	Enrich[c] Wheat	Enrich[c] Wheat
Control	Amb Cotton	Amb Cotton	Amb Cotton	Amb[c] Wheat	Amb[c] Wheat	Amb[c] Wheat

*test crop, not part of FACE experiment.
[a]SOC from original cotton plots sampled.
[b]soil air from original cotton plots sampled.
[c]SOC from wheat plots sampled.

Between experiments, the gas-distribution hardware was removed from the fields. Details of the cotton FACE experiments are described in Hendrey (1992), and the same methods were employed for the FACE wheat experiments. Field locations of replicate plots in the wheat experiments were different from those in the cotton experiments.

Figure 1 illustrates the petrochemically-derived CO_2 used to elevate concentration is depleted in ^{13}C relative to background atmosphere, and FACE plot plants acquire concordant depletion. Mixing this tank CO_2 ($\delta^{13}C \approx -37$ ‰) with open air (≈ -8 ‰) produces air above the FACE plots distinctively ^{13}C -depleted (≈ -18 ‰). Therefore the plants in the FACE plots have $\delta^{13}C \approx -38$‰ compared to plants in the Control plots with $\delta^{13}C \approx -27$ ‰. The relatively ^{13}C -depleted plant C in the FACE plots thus became a tracer with which to follow C entering SOC pools with initial $\delta^{13}C \approx -23$ ‰.

In the 1991 cotton FACE growing season, Leavitt et al. (1994) collected soils from two of the four plot replicates and analyzed stable-C isotopic composition of SOC, after first removing all plant fragments. The FACE SOC was depleted in ^{13}C by 1.9‰ relative to the Control soils ($\delta^{13}C$ =-24.15 ‰ and -22.24 ‰, respectively). Isotopic mass balance calculations indicated that 12% of the SOC at the end of the third year of experiment was derived from contemporary inputs. Fractionation of SOC pools by 6N HCl hydrolysis revealed similar ^{13}C depletion in the more active "supernatant" fraction (-22.52 ‰ and -21.09 ‰, respectively) and even ^{13}C-depletion in the recalcitrant "residue" fraction (-24.64 ‰ and -22.84 ‰, respectively). Because of the old, mean-radiocarbon ages of the residue fraction (2200 y BP), Leavitt et al. (1994) concluded that this was additional evidence that much, if not all, of the 12% new FACE SOC was "extra" rather than "replacement".

This 12% value for new SOC was an important quantitative measurement, even if it only represents balanced turnover rather than absolute increase of SOC.

In this paper we report (1) $\delta^{13}C$ of field-collected soil-air CO_2 from cotton plot soils (2) $\delta^{13}C$ results from continued sampling and analysis of SOC in cotton plots after cotton experiments ended, and (3) $\delta^{13}C$ results from the two years of FACE what experimentation.

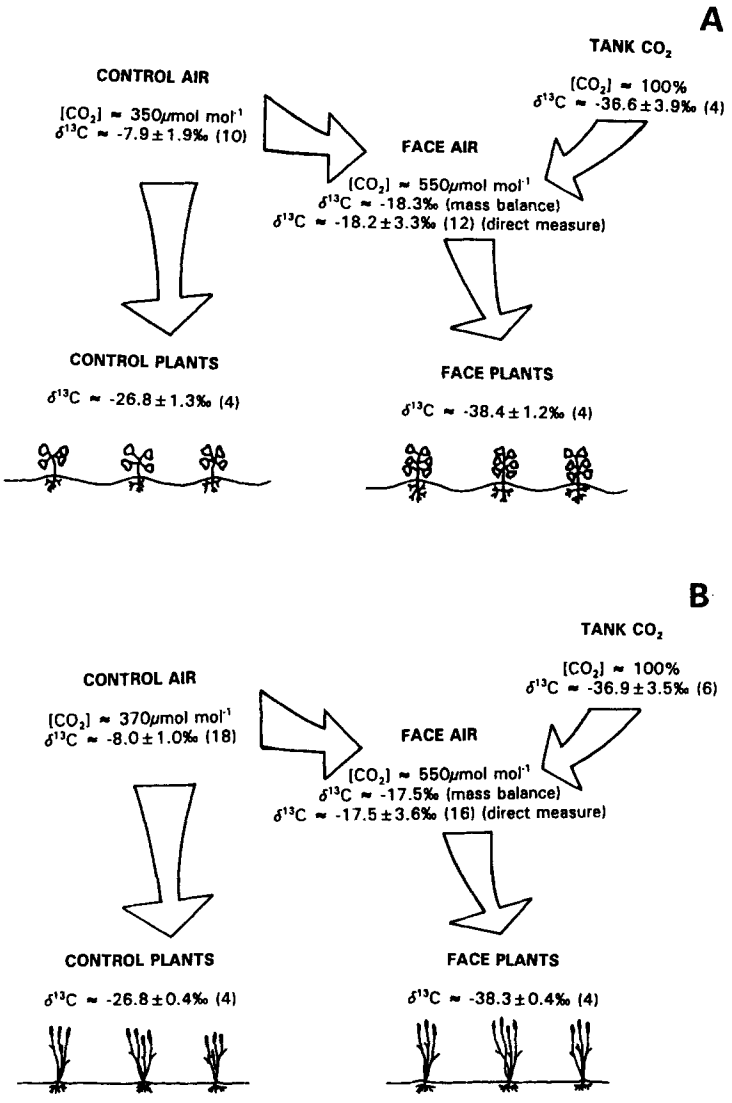

Figure 1. Stable-carbon isotopic composition ($\delta^{13}C$) in the air and plants of the cotton (**A**) and wheat (**B**) FACE experiments. The cotton isotope system diagram represents measurements from the 1991 growing season. The wheat diagram represents average tank CO_2 and air measurements from both the 1992–93 and 1993–94 growing seasons, and whole-tissue plant analyses are only from the end of the 1993–94 growing season, although they are consistent with $\delta^{13}C$ values of plant holocellulose measured on wheat from the 1992–93 experiment. The isotopic values represent mean ±ISD with no. of samples in parenthesis.

Methods

Soil air sampling

Table 1 summarizes the history of experimental and non-experimental crops growing on each plot and our sampling activities. Beginning in July 1992, soil air was sampled from the plots of the original FACE cotton experiments for isotopic analysis of the CO_2. Initially, we sampled with 2.5-L metal cans (17-cm tall), pushed ca. 4 cm into the soil of four original cotton plots (Control-FACE replicates 1 and 2). Two hours after each placement, gas samples were drawn from the cans into 3-L or 13-L evacuated flasks. Uncertainties about drawing open-atmosphere air into the flasks prompted later sampling with metal capillary tubes driven into the soil to 30 cm. Soil air was drawn with evacuated 125-mL flasks on October 21, and November 4 and 18, 1992. The final sampling procedure utilized permanently installed PVC pipes (35-cm long, 1.5-cm inside

diameter, valve on top) with a perforated bottom at a depth of 30 cm. Soil gas samples were drawn through the top into a 0.5-L evacuated flask at approximately biweekly intervals beginning on December 22, 1992.

The 1992–93 FACE wheat experiment plot locations did not interfere with the measurements because they were displaced northward, with no overlap, from the original cotton plot locations. To obtain CO_2 concentration in the soil atmosphere, capillary tubes were permanently installed around April 9, 1993, to sample gas at 10, 20, 30 and 40-cm depths adjacent to the PVC standpipes. A 1.5 to 2-mL soil air sample was taken through a rubber septum on top of each capillary probe and CO_2 concentration determined with an infrared gas analyzer (Nakayama and Kimball, 1988). Soil temperatures at 10-cm depth were obtained from the Arizona Meteorological Network's weekly summaries for the Maricopa Agricultural Center.

Soil sampling and laboratory preparation

Surface soils (0–30 cm) were sampled from original cotton FACE plots (FACE/Control replicates 1 and 2) on May 21, 1993. Surface soils (0–15 cm and in some cases 0–30 cm and 15–30 cm) were sampled from the wheat FACE replicate plots (1) prior to seed germination in the first 1992-93 experiment on December 17, 1992, (2) after the first experiment on May 27, 1993, (3) prior to the second 1993–94 experiment on November 30, 1993, and finally (4) after the second wheat experiment on June 2, 1994 (Table 1).

Soil samples were first sieved (1 mm) to remove large plant fragments and pebbles, and a 50–100 g subsample was acidified with cold 0.5-1 *N* HCl to remove the carbonate fraction. The acid was decanted and samples were then immersed in a concentrated NaCl solution ($\rho \approx 1.2$ g cm^{-3}) and stirred. Following settling of the heavier solids, the floating plant fragments were skimmed from the surface. This was repeated until no floating fragments remained, after which the soils were rinsed free of salt, oven-dried (70°C), and pulverized with a mortar and pestle. Recognizable plant and root fragments were physically removed from 1–5 g of these processed soil samples under a binocular microscope at 20×.

Isotopic analysis

Soil-air CO_2 was isolated on a vacuum line by freezing water vapor at -85°C, freezing CO_2 with liquid nitrogen at -195°C, and pumping away non-freezable gases. Nitrogen oxide was removed by circulating the CO_2 over hot (650°C) copper turnings for 15 minutes, after which the CO_2 was refrozen and the non-condensible N_2 evacuated. The purified CO_2 was analyzed mass-spectrometrically for $\delta^{13}C$[1] relative to the international PDB standard (Craig, 1957). Carbon yield was determined by manometric analysis of the recovered CO_2.

Soil subsamples (0.10–0.25 g) were combusted to CO_2 in quartz tubes at 900°C in the presence of copper oxide, silver foil and copper turnings (Boutton, 1991). SOC content was determined manometrically, and the CO_2 was analyzed mass-spectrometrically. Isotope ratio mass spectrometer precision is $\leq \pm 0.05$ ‰(± 1SD) for repeated analysis of the same CO_2 gas. During the last (1991) FACE cotton experiment (Leavitt et al., 1994), seven separate combustions and analyses of subsamples of one FACE soil sample produced a mean $\delta^{13}C$ of -23.40 ‰ (lSD=0.25 ‰) with a mean SOC content of 0.60% (lSD=0.03%). Seven additional runs with another soil standard gave a $\delta^{13}C$ precision (lSD) of 0.33 ‰ and SOC content precision of 0.06%. Additionally, two splits from each of 8 soils samples collected on May 21, 1993, from the wheat and cotton plots were processed and analyzed separately. The $\delta^{13}C$ mean difference of the pairs was 0.03 ‰ with a standard deviation of 0.35 ‰.

Results

Soil air

The $\delta^{13}C$ of soil air CO_2 increased during the fallow period from late Summer to Fall preceding the 1992–93 FACE wheat planting (Figure 2). Decreasing soil temperature from 31° (July 31) and 32°C (August 12) to 18° (November 4) and 16°C (November 18) would contribute to a decrease in microbial activity over the fallow period. Soil moisture ([weight water/weight dry soil] ×100) from September 9 through November 18 was very low (7.2-11.1 %) with no evidence for an increase or decrease. The amount of CO_2 recovered, however, was several typically 2–5 times greater than would be expected if there were no microbial respiration (i.e., if only aboveground air was in the soil pore space).

Following wheat planting, the soil CO_2 $\delta^{13}C$ rapidly declined with ^{13}C-depleted CO_2 inputs from wheat root respiration and microbial decomposition. The soil

[1] $\delta^{13}C$ (in ‰units) = $((^{13}C/^{12}C)_{sample}/(^{13}C/^{12}C)_{PDB} - 1) \times 1000$

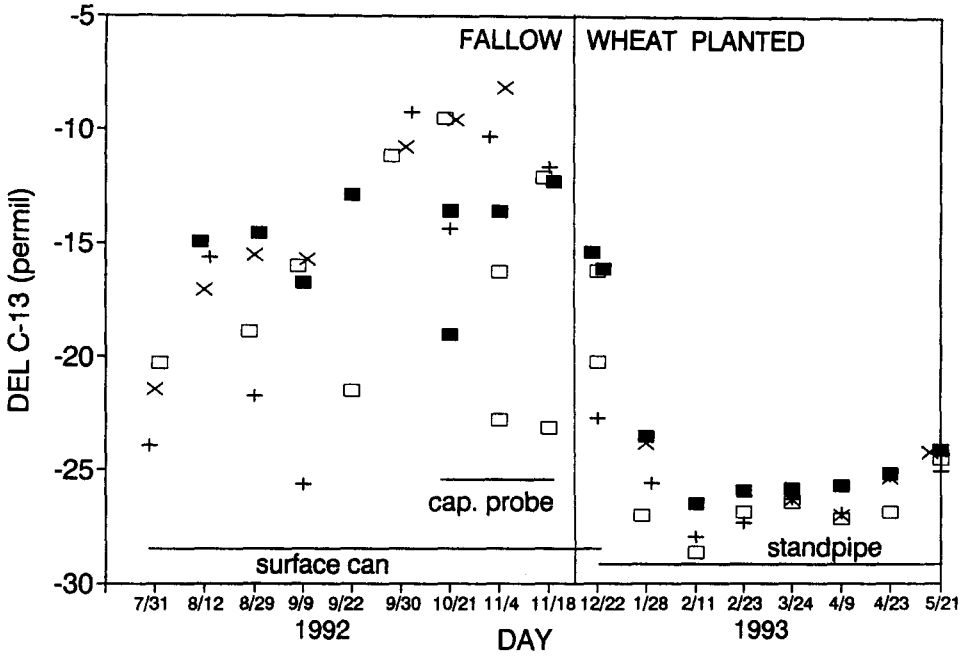

Figure 2. The $\delta^{13}C$ of respired CO_2 collected in the field. Three different methods were used: inverted can samples, capillary probe samples, and finally samples from PVC standpipes. Plot designations are "FACE 1" (CO_2 -enriched plot 1) = □, Control 1= ■, FACE 2 = +, Control 2 = X.

temperature also increased from ca. 9°C to 21°C from December 22, 1992, to April 23, 1993, respectively. Soil moisture levels increased after planting due to irrigation and January-February rainfall. Likewise the CO_2 recovered in the 125-mL flasks was typically 20–70 times greater than expected if there were no respiration in the soils. That CO_2 recovery represents concentrations $\geq$ 2%, and consistent with the direct CO_2 concentration measurements from 30- and 40-cm depths sampled in April and May 1993 (Fig. 3).

The average soil-air fallow-period $\delta^{13}C$ values indicate that the FACE plots (mean=-16.72‰, 1SD = 5.32 ‰, n = 19) were 2.4 ‰ more negative (p <0.05 : t-test) than the Control plots (mean = -14.36 ‰, 1SD = 3.38%, n =17). A more conservative test with only the most numerous fallow-period $\delta^{13}C$ data (3-L flasks from the inverted metal can) indicated the FACE plot soil air tended to be only 0.63 ‰ more negative than the Control (not significant: paired t-test).

After planting, the FACE soil CO_2 still averaged 1.3 ‰ more negative than the Control (-26.87 and -25.53 ‰ for FACE and Control, respectively, p<0.01: paired t-test). This result is notable in light of the likely large inputs of ambient wheat root-respired CO_2 whose isotopic composition should have been identical in cotton FACE and Control plots. The CO_2 contributed from microbial decomposition of older C from the previous cotton FACE experiment must have likewise increased to maintain a large isotopic difference.

Despite isotopic differences, the average quantities of CO_2 recovered in the soil air samples for both the fallow period and the period after wheat planting were not significantly different between FACE and Control plots.

SOC $\delta^{13}C$-cotton experiments

The $\delta^{13}C$ signal persisted in the cotton soils sampled in May 1993, ~ 1.5 years after the end of the FACE experiments. The difference of 1.9 ‰ between Control (-23.9 ‰) and FACE (-25.8 ‰) is identical to that of October 1991. The absolute $\delta^{13}C$ values of the 1993 FACE and Control SOC, however, were ~ 1.5 ‰ more negative on May 21, 1993, than October 1991. This may be a consequence of the intervening two wheat crops (Table 1) growing on those plots: 1991–92 (without a FACE experiment) and 1992–93 (experiment in progress). In fact, the SOC content in May

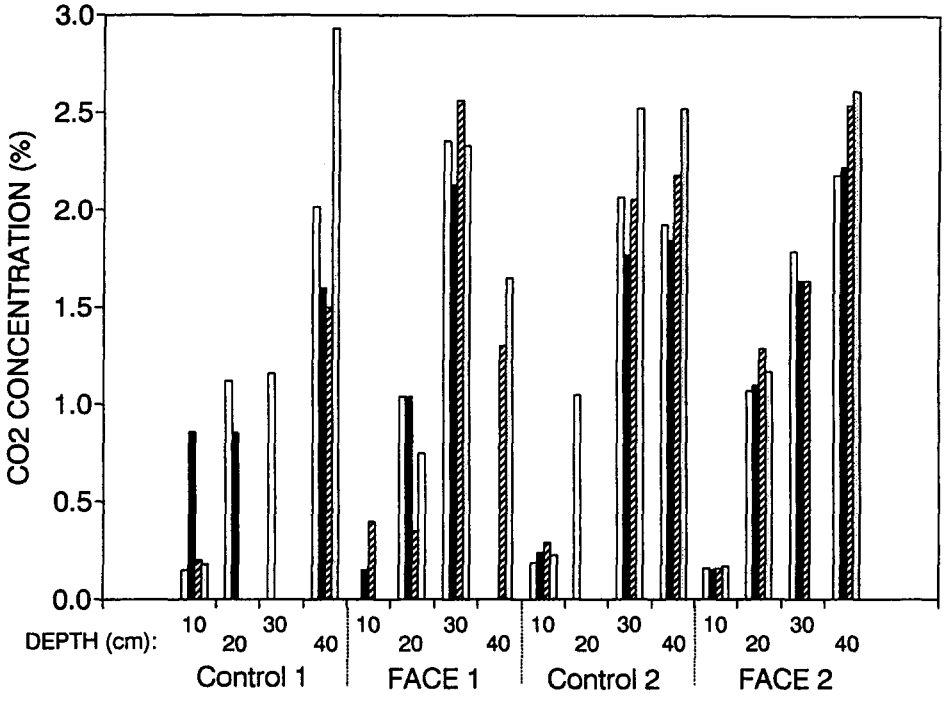

Figure 3. CO_2 concentrations measured at different soil depths, on April 9, 1993 (open bars), April 2l), 1993 (solid), April 29, 1993 (diagonal pattern), and May 21, 1993 (shaded). Missing bars indicate no analysis.

1993 was 0.73–0.76%, compared to SOC contents of 0.56–0.64% in October 1991.

SOC $\delta^{13}C$ wheat experiments

The $\delta^{13}C$ and SOC content for the wheat FACE experiments measured at the beginning and end of each growing season are given in Figures 4A and 4B, respectively. The nearly identical FACE and Control values on May 17, 1992, (average of one sample each from replicates 1 and 2) indicate homogeneity of isotopic and carbon content prior to the beginning of experimentation. $\delta^{13}C$ from two sets of samples at the end of the first growing season, May 1993, are presented in Figure 4A. The first is from 0–30 cm depth soils sampled on May 21, 1993, and the second from 0–15 cm depth sampled on May 27. These values are averages of one sample each, from replicates 1 and 2. The data are fairly consistent between the two samplings, and both show $\delta^{13}C$ of FACE soils more negative by 0.6 and 0.2 ‰, respectively. The SOC content (Figure 4B) did not differ between FACE and Control, but the 0–15 cm samples tended to have more SOC than 0–30 cm.

Samples for November 30, 1993, were analyzed from the 0–15 cm depth at all four replicates (Figure 4B), but the average results for 0–15 cm of replicates 1 and 2 alone are also shown for better comparison with soil analyses from the other samplings. The $\delta^{13}C$ of FACE SOC is ~ 1 ‰ more negative than the Control for both sets of data, and the FACE soils tended to have more SOC.

Soil samples from 0–15 cm in June 1994 continued to show a pronounced difference between FACE and Control of 1.1 ‰ for the average of all four replicates and 0.7 ‰ for two replicates (1 and 2). The sensitivity of these isotopic signals is highlighted by the SOC contents, which showed little difference between FACE and Control. For November 1993 and June 1994, the 15–30 cm soils showed little $\delta^{13}C$ difference between FACE and Control although the FACE SOC content tended to be higher than the Control in these deeper soils.

Discussion

Soil air

The results show relatively ^{13}C-depleted CO_2 respired from the cotton FACE plot locations (compared to Con-

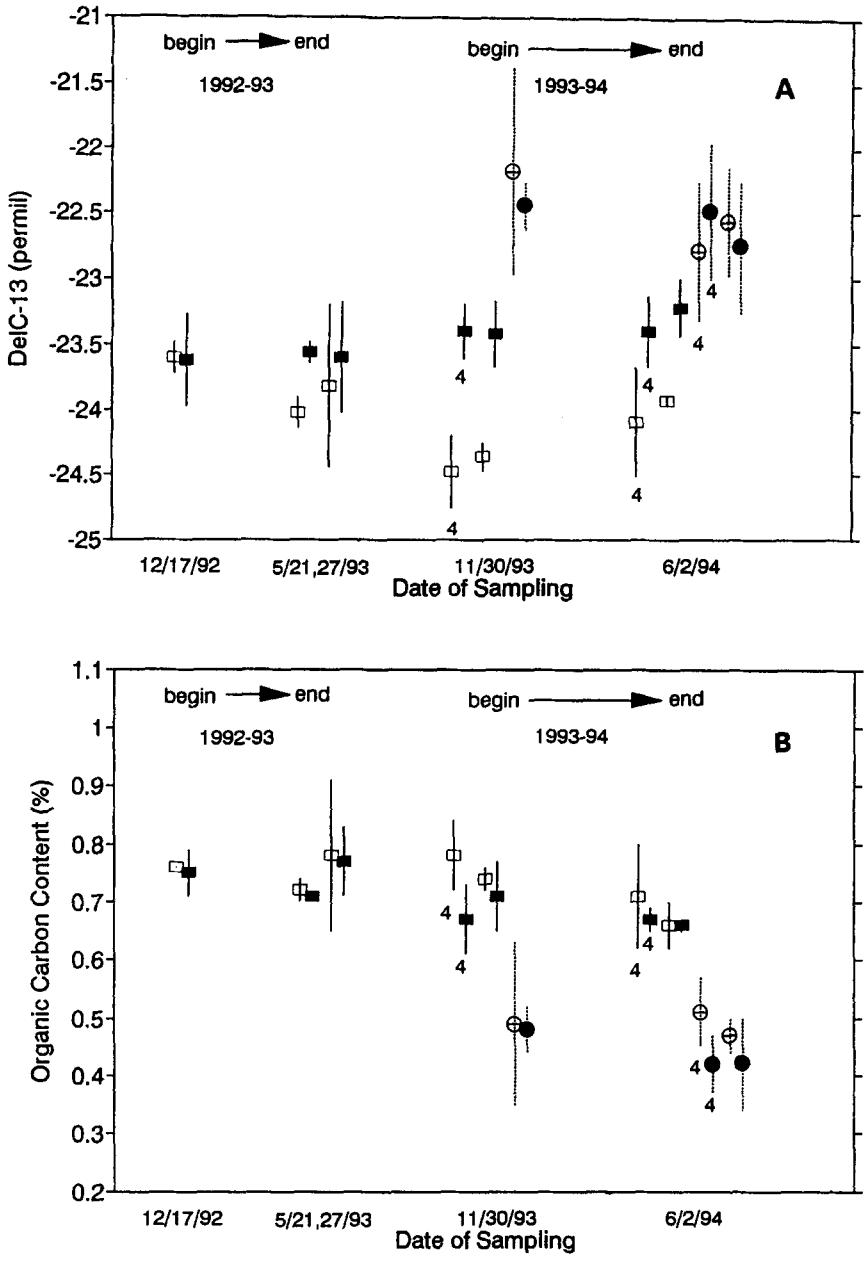

Figure 4. $\delta^{13}C$ (**A**) and SOC content (**B**) in soils sampled before and after the 1992–93 and 1993–94 wheat FACE experiments. Most open squares are the average of 1 surface soil sample (0–15 cm) from each of two CO_2 enriched plots (i.e., n=2 from replicates 1 and 2) and the solid squares are the average of 1 soil sample from each of two corresponding Control plots (0–15 cm). Exceptions include those labeled with a "4" which are the averages of 1 soil analysis from each of all four replicate plots 1, 2, 3, and 4. Also, the points for 5/21/93 are actually from soil samples from a depth of 0–30 cm. Open (CO_2-enriched) and filled (control) circles represent analysis of soils at a depth of 15–30 cm with same values of n as described above. Error *bars* are ± ISD.

trol plots) during the post-experiment fallow period. This relative depletion continued through the following wheat crop grown on the plots, despite large CO_2 contributions from wheat root respiration (and decay of wheat plant matter and exudates) that would have been isotopically indistinguishable. Thus, new SOC

added during the FACE cotton experiments must be a major substrate for microbial decomposition.

This pattern emerges despite fallow-period $\delta^{13}C$ values (Figure 2) being typically 5–10 ‰ less negative (^{13}C-enriched) relative to the previously-measured cotton SOC values of -22 ‰ in Control and -24 ‰ in FACE. Although preferential decomposition of ^{13}C-enriched SOC might play some role, such sizable ^{13}C-enrichment suggests a significant aboveground air component ($\delta^{13}C \approx$ -8 ‰), perhaps resulting from either reduced microbial activity or substantial aboveground air collected inadvertently during the gas sampling. Additional isotopic inconsistencies associated with sampling may derive from theoretical isotopic differences between "soil CO_2" (CO_2 in void space of soils sampled by capillary tube and standpipe) and "soil-respired CO_2" (CO_2 emitted from soils and collected over time continuously in a surface container) as described by Davidson (1995). Cerling et al. (1991) found evidence that "soil CO_2" may be about 4 ‰ less negative than "soil-respired CO_2" because of isotopic fractionation during CO_2 diffusion from the soil.

Isotopic estimation of SOC input

For **cotton** the FACE C input required to lower the $\delta^{13}C$ of the FACE, SOC by 1.9 ‰ relative to Control can by estimated by isotopic mass balance (Balesdent et al., 1988) using the following equation:

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

If organic C from the enriched cotton plants was the only new C added to the SOC, then $f_{soil\ original}$ (the fraction of "old" C in the current SOC) is equal to 1-f_{input} (where f_{input} is the fraction of new FACE carbon in the current SOC pool). Using -38.4 ‰ as $\delta^{13}C_{input}$ (cotton composition, Figure 1), -23.9 ‰ as $\delta^{13}C_{soiloriginal}$ (May 1993 Control SOC), and -25.8 ‰ as $\delta^{13}C_{FACE\ soil}$ (May 1993 FACE SOC) in the preceding equation, f_{input} equals 13%. This, of course, assumes the $\delta^{13}C_{soil\ original}$ is -23.9 ‰, whereas it was actually -22.2 ‰ at the end of the 1991 FACE cotton season. However, it is the FACE-Control difference (not absolute value) which is key to the analysis, so that with an additional assumption that both FACE and Control SOC shifted the same $\delta^{13}C$ amount from 1991 to May 1993, C input may be estimated from the isotopic difference. Realistically, the f_{input} represents the amount of new FACE plant carbon alone necessary to depress $\delta^{13}C$ of the FACE SOC from pre-experiment values. It is thus a quantitative estimate of C turnover, but lacking an isotopic tracer signal in the Control plots, we cannot resolve the extent to which C additions to FACE exceed Control.

For **wheat**, the $\delta^{13}C$ values from FACE replicates 1 and 2 for 0–15 cm were used to calculate f_{input}. A value of -38.3 ‰ was used for $\delta^{13}C_{input}$. The calculations indicate 2% new SOC input at the end of the first wheat experiment, 6% at the beginning of the second wheat experiment, and 5% by the end of the second experiment. The 5–6% input is about one-half that calculated for the cotton FACE experiment from the 1991 soil isotopic analyses. The difference could be a consequence of the extra year of cotton compared to wheat experimentation (1991 was the third cotton FACE growing season), or perhaps greater cotton-root biomass (Prior et al., 1994; Wechsung et al., 1995).

The isotopic signal is weaker at the end of the first experiment than 6 months later. This was attributed to the intervening period of warm temperatures and summer rainfall aiding decomposition and incorporation of wheat residues into the SOC (preparation of May 1993 soils would have mechanically removed such residue). The isotopic composition of 15–30 cm SOC was much less negative than 0–15 cm, but there was little difference between FACE and Control. This is consistent with cotton FACE findings (Leavitt et al., 1994) of no isotopic signal in the deeper layers (30–60 cm).

Interpretation of all findings must be tempered by recognition of likely heterogeneity in the $\delta^{13}C$ and SOC content of the soil system, and greater insight might be gained by further analysis of physical and chemical separations of SOC fractions. The isotopic labeling appears robust enough for multi-year sampling needed to study SOC pools with extended equilibrium times.

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