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Stable-carbon isotopes and soil organic carbon in wheat under CO_2 enrichment

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Summary

- Stable-carbon isotopic tracers were enlisted in 1996 and 1997 wheat (*Triticum aestivum*) free-air CO_2 enrichment (FACE) experiments to detect entry of new C into soil organic carbon (SOC) pools. Any enhanced soil inputs might mitigate rising atmospheric CO_2.
- The CO_2 used to enrich FACE plots (to ambient +190 μmol mol^{-1}) resulted in ^{13}C-depleted wheat relative to ambient plants and the native SOC. To trace new C in control plots C_4-plant-derived exotic soils were placed into subplots in high-N FACE and control treatments, and a $^{13}CO_2$ gas tracer was pulsed to subplots in high-N control replicates.
- Under high-N, isotopic mass balance showed 6% ($P = 0.003$) and 5% ($P = 0.04$) new C in 0–15-cm and 15–30-cm FACE SOC, respectively, after 2 yr. Results from the C_4-soil subplots were ambiguous, but the $^{13}CO_2$ tracer induced a SOC $\delta^{13}C$ increase ($P = 0.08$) at 15–30 cm in control-high N consistent with 6% new C.
- We infer *c.* 3% $year^{-1}$ (30–40 g C m^{-2} yr^{-1}) SOC turnover in surface soils at high-N under both ambient and elevated CO_2. The $^{13}CO_2$-tracer result, however, is less reliable because of lower significance, fewer replicates and heterogeneous isotopic distribution within plants.

Key words: wheat, carbon isotopes, carbon sequestration, global change, free-air CO_2 enrichment (FACE), soil organic carbon, SOM.

Introduction

The effects of rising atmospheric CO_2 concentrations on belowground processes, including soil carbon storage, have yet to be fully revealed (Curtis *et al.*, 1994; Canadell *et al.*, 1996), and it is becoming more evident that experimentation with different vegetation and soil types is necessary to better anticipate soil response to future high CO_2 environments. Free-air CO_2 enrichment (FACE) experiments provide the opportunity to examine many aspects of elevated CO_2 effects on ecosystems, and particularly the response of soil organic carbon (SOC) pools under realistic field conditions. Additionally, stable-carbon isotopic tracers have been successfully used in several recent CO_2-enrichment experiments (Leavitt *et al.*, 1994, 1996; Hungate *et al.*, 1997; Nitschelm *et al.*, 1997; Torbert *et al.*, 1997; Van Kessel *et al.*, 2000a) to estimate carbon partitioning and turnover in soils.

Two successive FACE experiments were conducted at The University of Arizona's Maricopa Agricultural Station to investigate $CO_2 \times N$ interactions in wheat. Planting was in December 1995 and December 1996, and the growing season was completed by May of each following year. The stable-carbon isotopic composition ($\delta^{13}C$ in ‰ = $[[(^{13}C/^{12}C_{sample} \div {}^{13}C/^{12}C_{PDB}) - 1] \times 1000]$, where PDB carbonate is the isotopic reference standard) of the commercial CO_2 used to elevate concentrations was very distinct (−40‰) from background

air (−8‰). The addition of about 190 μmol mol^{-1} of this commercial CO_2 to the high-CO_2 plots provided the ^{13}C-depleted tracer to estimate new SOC influx. The isotopic tracing method works best when the isotopic difference between SOC and plants contributing to SOC are greatest. The method is less effective in ambient CO_2 plots because the $\delta^{13}C$ of C_3 plants is typically only a few ‰ different from that of the SOC of existing soils developed under C_3 plants. Lacking such an isotopically distinctive tracer in the control plots, we employed two methods to estimate carbon entry into those soils: a $^{13}CO_2$ tracer to which subplots were exposed (a 'first' in FACE experiments); and subplots of exotic soil in which the SOC was much more ^{13}C-enriched relative to the wheat plants than was the native SOC. The objectives were to quantify the input of new C derived from the wheat crops to SOC, and to assess the influence of elevated CO_2 on this input.

Methods

General

Spring wheat (*Triticum aestivum* L. cv. Yecora Rojo) was grown at the University of Arizona Maricopa Agricultural Center in a large farm field, within which were situated four replicated plot pairs comprised of 25-m diameter enriched and control plot rings. These rings were positioned to avoid overlapping any locations occupied by plots in previous cotton and wheat FACE experiments. The control plots had daytime background ambient CO_2 concentrations of 360–370 μmol mol^{-1}, whereas the enriched plots were maintained at approximately ambient +190 μmol mol^{-1} for 24 h d^{-1} via a computer-controlled CO_2 distribution system. The experimental CO_2-enrichment system has an excellent record gained from inaugural operation with cotton $CO_2 \times H_2O$ FACE experiments (details in Hendrey, 1992; Dugas & Pinter, 1994), followed by initial FACE wheat $CO_2 \times H_2O$ experiments (Kimball *et al.*, 1995). In this $CO_2 \times N$ experiment, each plot was divided into a high and low N treatment in a strip-split-plot design (Kimball *et al.*, 1999). Added fertilizer N was 5 times greater in high-N (350 kg N ha^{-1}) than low-N (70 kg N ha^{-1}) treatments in the first year, but over 20 times greater in high-N (350 kg N ha^{-1}) than low-N (15 kg N ha^{-1}) in the second year (except the control low-N plot in replicate 3 that received *c.* 135 kg N ha^{-1}). Abundant water was provided by subsurface microirrigation with a *c.* 23-cm 'drip' tube depth, 50-cm horizontal spacing of tubes, and emitter spacing at 30 cm along the tubes. Irrigation took place after 30% of available water in the root zone was depleted, with cumulative amounts ranging between *c.* 55 and 70 cm depending on treatment and season. The enriched ('FACE') plots employed blowers to predilute the CO_2 before release into the plots. Unlike previous experiments, our Control plots in these wheat experiments had identical blowers (Pinter *et al.*, 2000).

C_4 soil subplots

Small subplots of two different soils developed under C_4 plant vegetation (^{13}C-enriched compared with the *in situ* soils) were installed in the high-N side of the FACE and Control plots in replicates 1 and 2. One soil ('Texas') was obtained from near Temple, Texas, USA, in a prairie environment dominated by C_4 grasses, and the second ('Randolph') was collected from a golf course undergoing renovation in Tucson, Arizona, USA, where C_4 Bermuda grass (*Cynodon dactylon*) had been grown for several decades.

The $\delta^{13}C$ of SOC in these soils was −17.1 ± 0.6‰ and −20.3 ± 0.2‰, for Texas and Randolph, respectively (SOC content of 2.0 ± 0.3% and 0.9 ± 0.2%, respectively). Surface *in situ* soils at Maricopa have a $\delta^{13}C$ of *c.* −23‰ and a lower SOC content of 0.6–0.7%. The Texas soil was fine-grained, Houston Black series, Udic Haplusterts (24% sand, 33% silt, 43% clay), whereas the Randolph soil was fairly coarse-grained, Nickel-Latene–Cave Association, thermic semiarid (68% sand, 22% silt, 10% clay). The texture of the local Maricopa *in situ* reclaimed Trix clay loam was intermediate between the exotic C_4 soils with 27–45% sand and 27–40% clay, classified as a fine loamy, mixed (calcareous), hyperthermic Typic Torrifluvent (Post *et al.*, 1988).

Soils were well mixed in a large (cement-)mixing device and distributed to buckets designated for each field position. SOC content and $\delta^{13}C$ were determined on splits from each bucket before soils were distributed to the field. Texas and Randolph soil subplots (*c.* 1200 cm^2 each and 15-cm deep) were installed immediately after planting, and seeded by hand. Before filling each excavated subplot with these soils, they were lined with several layers of woven plastic shading material to maintain the exotic soil separate from the native soil yet permit water movement. Each subplot included two rows of wheat. At the end of the first experiment two 12-mm cores were taken for analysis from each subplot between the two rows of wheat from the 0–15 cm interval. At the end of each growing season, the soils were removed from the field, mixed, sampled for analysis and dried. In between experiments, the soils were stored in separate containers and then distributed to the same field position occupied in the 1995–96 experiment.

Pulsed $^{13}CO_2$ tracers

An isotopic tracer was applied to subplots (1 m^2) within the high-N side of control replicate plots 1 and 2 by means of the same chambers used for whole-canopy gas exchange measurements (Garcia *et al.*, 1998; Brooks *et al.*, 2001). The chambers were installed over the soil subplots, and a pure (99%) $^{13}CO_2$ tracer (contained in a 250-L cylinder as a 17.5 : 1 $N_2 : CO_2$ gas mixture) was bled into the airflow entering the chambers. The bulk airflow was equivalent to about three air exchanges in the 1-m^3 chamber per min, and the $^{13}CO_2$ was added at a rate calculated to induce a 10–12‰

^{13}C enrichment in bulk plant isotopic composition relative to the untreated plants in the control plots at the end of each growing season. The tracer was admitted at an average rate of 40–60 cm^3 min^{-1} for *c.* 35 h on 6 d (2 Feb, 6–8 Mar, 15–16 Mar) in the 1995–96 experiment, and for *c.* 40 h over 7 d (12–13 Feb, 7–8 Mar, 5–7 Apr) in the 1996–97 experiment. Air samples were periodically taken with evacuated glass flasks to confirm the large ^{13}C-enrichment of the chamber air (*c.* +200 to +400‰) during the application periods. The high cost of the $^{13}CO_2$ prevented a plot-wide application of the tracer, but use of the chambers to input the tracer over a very small fraction of the growing season allowed most of the plant growth to occur under 'free-air' conditions.

Sampling and analysis

Air Air samples were collected with 2-l and 3-l evacuated flasks approx. every 2 wk of the growing season from the center of ambient and enriched plots in replicates 1 and 2. In 1995–96, the air was drawn directly from the plot into the flasks, whereas in the second year an integrated sample was obtained by pumping air over 10 min into a 10-l mylar balloon and taking the flask sample from well-mixed air within the balloon. CO_2 was isolated from the flasks by cryogenic trapping on a laboratory vacuum line system. The purified CO_2 was analyzed on a Finnigan (Finnigan MAT, Bremen, Germany) Delta-S mass-spectrometer to determine $\delta^{13}C$ with respect to the internationally accepted VPDB (Vienna-PDB carbonate) reference standard (Craig, 1957; Coplen, 1995). Periodically, commercial tank CO_2 samples were taken with 10-ml evaculated vials and similarly processed and analyzed to quantify variability of the commercial source.

Plants Plant samples were collected from all four replicates of the four treatments, dried and ground to 20-mesh. Whole plants from the end of the 1995–6 growing season, and green leaves and stems from day-of-year (DOY) 105 *c.* 1 month before maturity of the 1996–7 growing season were analyzed. Two to 4 mg of each sample were combusted in a recirculating micro-combustion system in the presence of excess O_2. The CO_2 was isolated cryogenically and analyzed mass-spectrometrically.

Soils The *in situ* soils were collected with a hand auger at two locations from three depths (0–15 cm, 15–30 cm, 30–60 cm) in all replicates (1, 2, 3, 4) and treatments (high CO_2-high N; high CO_2-low N; low CO_2-high N, low CO_2-low N) before and after each growing season. These soils, and those from the C_4 plots and ^{13}C-tracer chamber positions, were initially sieved to remove rock grains and plant fragments larger than 1 mm. Inorganic carbonates were dissolved with 1N HCl, and floating plant fragments were skimmed from the surface of the mixture. A concentrated NaCl solution (ρ c. 1.2 g cm^{-3}) was subsequently used to float persisting plant fragments, which were likewise skimmed. Soils were then rinsed, dried and pulverized with a mortar and pestle. These carbonate-free soils were examined at 20× magnification and remaining recognizable plant fragments were manually removed. The SOC can therefore be considered to be dominantly mineral-associated organic matter with some fine particulate organic matter (Cambardella & Elliott, 1992). Splits of 100–200 mg of soil sample with CuO powder and Ag foil were combusted (900°C for 2 h, 650°C for 2 h) in evacuated, sealed quartz tubes (after Boutton, 1991). From cryogenically separated CO_2, SOC content was determined manometrically and $\delta^{13}C$ was analyzed mass-spectrometrically.

Statistical analysis

Comparisons among treatment results for the same time period were done with one-way ANOVA or Student *t*-tests. Comparisons of treatment results over successive time periods were made with repeated measures ANOVA.

Results and Discussion

Air and plants

Figure 1 summarizes the overall isotopic composition of various aboveground components of the 1995–6 and 1996–7 FACE wheat systems. The $\delta^{13}C$ of commercial CO_2 was strongly ^{13}C-depleted and stable throughout the experiment between −40 to −41‰. Flask sampling results suggest daytime FACE air was 8–9‰ ^{13}C-depleted relative to control plot air, but isotopic composition of FACE air calculated from mass balance with CO_2 concentrations and measured $\delta^{13}C$ of tank gas and control air indicates FACE air was *c.* 10–11‰ more ^{13}C-depleted. This discrepancy could be a consequence of greater heterogeneity within the FACE rings resulting from imperfect mixing, and near-instantaneous sampling that does not capture the bulk plot isotopic composition well. The FACE air $\delta^{13}C$ mean from 1996 to 97, derived predominantly from mixing-balloon samples, was closer to the mass-balance estimate than was the grab-sample measurement mean from 1995 to 96. The consistent difference of 11–12‰ between FACE and control wheat plants supports the mass balance-estimated FACE $\delta^{13}C$ as being more representative of the average isotopic composition of air in the FACE plots. The control air $\delta^{13}C$ values of −8.4 to −8.8‰ are somewhat ^{13}C-depleted relative to global background air values of −7.9 to −8.0‰ measured at Mauna Loa as part of NOAA's flask sampling network (FTP://ftp.cmdl.noaa.gov/ccg/co2c13/flask/complete/mLo.co2c13). The difference is probably largely a consequence of both general continental effects (respired CO_2) on background air at Maricopa and biasing by N_2O (same mass 44 as $^{12}C^{16}O_2$) not removed before mass-spectrometric analysis that reduces the air $\delta^{13}C$ values by *c.* 0.3–0.4‰ (Keeling *et al.*, 1979). This supports negligible contamination effects of FACE air on control plots.

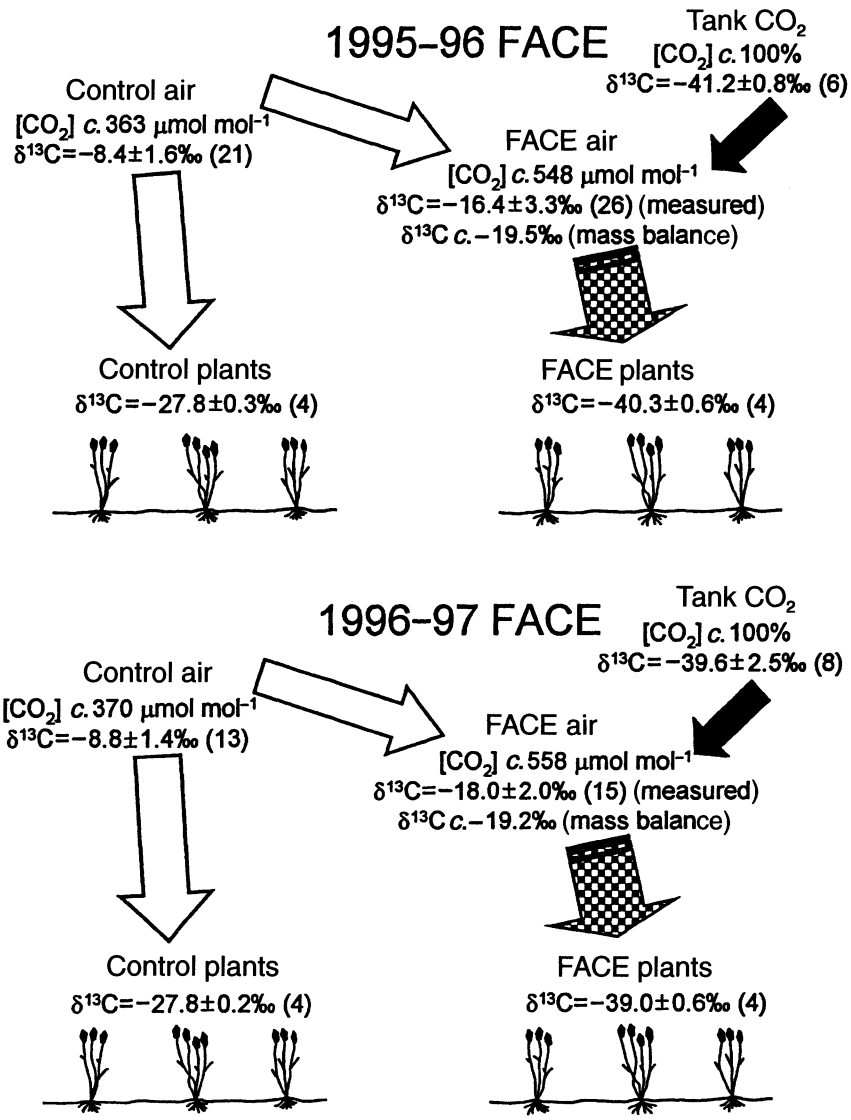

Fig. 1 Air and plant FACE wheat system $\delta^{13}C$ values for 1995–6 and 1996–7. Air and tank CO_2 were sampled approximately biweekly from the center of replicate 1 and 2 rings. CO_2 concentrations are daytime growing season means. Plant $\delta^{13}C$ represents the mean of bulked samples from each of the four high-N plots for end-of-season whole plants in 1995–6 and average green leaves and stems from day-of-year (DOY) 105 in 1996–7. Low-N plant $\delta^{13}C$ means were not distinguishable from high-N (Table 1). Numbers in parentheses are the total number of samples in the case of the air measurements and the number of replicate treatments included in the mean of the plant measurements. Open arrows, background air; solid arrows, pure commercial CO_2; checkered arrows, mixed background; and commercial CO_2 above FACE plots.

Table 1 $\delta^{13}C$ mean and standard deviation composition of wheat plants in the 4 replicates of each treatment (each replicate sample consisted of several plants bulked together). Values vertically with same letter designation are different

	FACE		Control	
5/96 End of season-whole plant				
High-N	−40.3‰	a**	−27.84	b**,c*
	0.61‰		0.28	
Low-N	−39.79	d*,e*	−28.13	f**
	0.47		0.18	
5/97 DOY 105-green leaves				
High-N	−39.52	g	−28.58	c*,h**
	0.97		0.31	
Low-N	−39.08	e*	−28.35	i**
	0.34		0.57	
5/97 DOY 105-stems				
High-N	−38.49	a**,g	−27.01	b**,h**
	0.41		0.19	
Low-N	−38.70	d*	−27.04	f**,i**
	0.58		0.26	

One-way ANOVA comparisons indicate *F*-test significant differences between high-N and low-N on each date/material, and high-N (or low-N) between dates by common letter ($P < 0.10$) and designation *($P < 0.05$), **($P < 0.01$).

The complete summary of wheat isotopic composition for all treatments is given in Table 1. There are some significant differences between years, with 1995–6 whole-plant material frequently ^{13}C-depleted compared with the 1996–7 stem counterparts, but enriched relative to 1996–7 control leaves. In 1996–7, the leaves were always more ^{13}C-depleted than the stems. There were no isotopic differences between high-N and low-N in the same year for the same CO_2 treatment.

Soil isotopes in native soil

The average $\delta^{13}C$ of soil organic carbon from three depths in all treatments before and after each experiment is plotted in Fig. 2. The low-N treatment 30–60 cm soils were not analyzed at the end of the first season (May 1996), and none of the 30–60 cm soils from December 1996, before the second season, were analyzed. The very high variability at 15–30 cm in December 1996 for all treatments is quite distinct, and perhaps some consequence of heterogeneous mixing of shallow and deep soils from the plowing/planting regime at the beginning of the second season, immediately before the soils were augered. Interestingly, the 0–15-cm plots in December 1996 showed the lowest isotopic variability of any of the time periods.

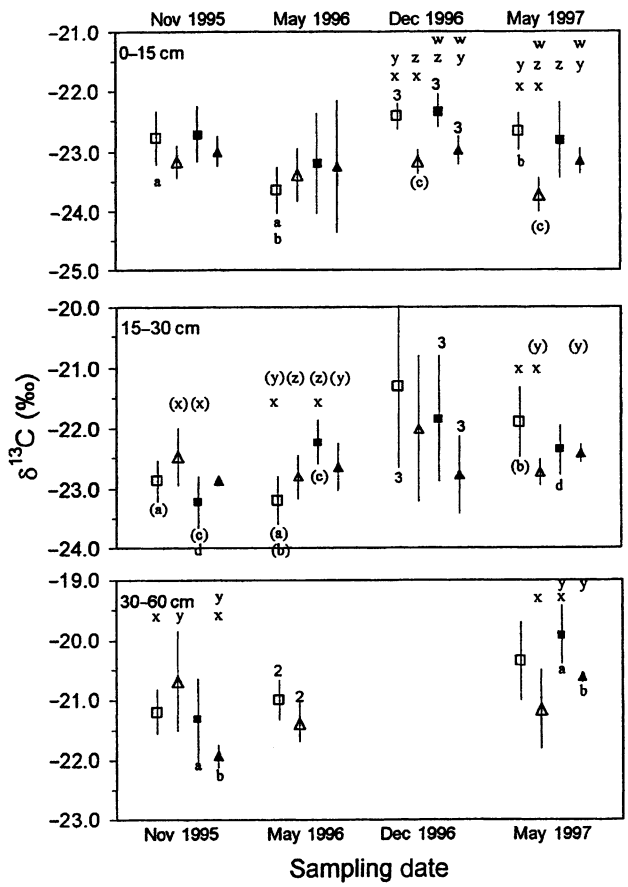

Fig. 2 Mean $\delta^{13}C$ of four replicates of all treatments measured throughout the 2-yr experiment at 0–15 cm, 15–30 cm and 30–60 cm. The November 1995 samples were augered immediately before planting of the first experiment; December 1996 samples were augered after planting but before emergence. Vertical bars are ±1 SD (usually equivalent to ±2 SE). If fewer than four replicates are in mean, a number (2 or 3) on the vertical bar indicates number of replicates. Statistical comparisons among treatments for a given date were done with *t*-tests, and values indicated with same letter ($P < 0.05$) or letter in parenthesis ($P < 0.10$) above vertical bar are significantly different. Statistical comparisons for same treatment between dates were made with repeated measures ANOVA, and values with same letter ($P < 0.05$) or letter in parenthesis ($P < 0.10$) below vertical bar are significantly different. Control, high N (open squares); FACE, high N (open triangles); control, low N (closed squares); FACE, low N (closed triangles).

At the beginning of the experiment SOC $\delta^{13}C$ of the 0–15 and 15–30-cm soils was quite uniform, with the exception of the 15–30-cm FACE-high N ('FACE-high') and control-low N ('control-low') locations that tended to be different ($P = 0.06$). There were much greater differences at 30–60 cm among plot locations. This suggests minimal isotopic heterogeneity in the surface layers before the first experiment, perhaps because of activities (including plowing/disking) during field preparation, as noted in soils from previous FACE wheat $CO_2 \times H_2O$ experiments (Leavitt *et al.*, 1997).

The overall isotopic trajectory of all treatments at 0–15 cm (Fig. 2) seems to show somewhat lower $\delta^{13}C$ means in May 1996, higher means in December 1996, and a decline thereafter, with some of these changes significant. General isotopic enrichment thought to be associated with decomposition (for example Dzurec *et al.*, 1985; Wedin *et al.*, 1995) may contribute to part of the pattern. The early part of the trajectory may be impacted by residues from the oat crop that immediately preceded the experiment by contributing carbon of different isotopic composition and turnover rates. Power *et al.* (1986) found large differences in timing of decomposition of different crops, and Martens (2000) showed very rapid decomposition of oat residues relative to other plants such as prairie grasses that would probably be most similar to wheat. Such shifting $\delta^{13}C$ trajectories were not seen in soils from the previous wheat FACE experiment (Leavitt *et al.*, 1996).

By the end of the experiment in May 1997, at 0–15 and 15–30 cm the $\delta^{13}C$ of FACE-high SOC was ^{13}C-depleted compared with control-high ($P = 0.003$ and $P = 0.04$, respectively). This is consistent with a significant fraction of new ^{13}C-depleted organic matter from the FACE plots entering the SOC pools. The low-N plot SOC at 0–15 and 15–30 cm in May 1997 shows no difference between FACE and control conditions ($P = 0.34$ and $P = 0.79$, respectively). However, the FACE-low treatment is ^{13}C-depleted relative to control-low at 30–60 cm ($P = 0.03$). There were no FACE-control isotopic differences at the end of the first growing season but there were (at 0–15 cm) in December 1996 before the beginning of the second growing season ($P = 0.006$ for high-N and $P = 0.04$ for low-N). This outcome is similar to that seen in the previous FACE wheat $CO_2 \times H_2O$ experiments, for which it was suggested that the 7-month lag between the end of the

first growing season and the beginning of the second permitted decomposition of crop residues and incorporation of products into the subsequently analyzed SOC pools (Leavitt *et al.*, 1996). It is also consistent with time lags implicit in decomposition of various crops (Power *et al.*, 1986; Martens, 2000).

Comparing $\delta^{13}C$ values on November 1995 and May 1997, only control-low 15–30 ($P = 0.04$), control-low 30–60 ($P = 0.02$) and FACE-low 30–60 cm ($P = 0.002$) exhibited significant differences (ANOVA repeated measures F-tests). In all three of these cases, the SOC $\delta^{13}C$ in May 1997 is ^{13}C-enriched compared with November 1995.

Soil SOC

There was no significant difference in SOC among the treatment locations before the beginning of the first experiment at any of the depths with the exception of FACE-high at 30–60 cm and FACE-low at 30–60 cm that tended to be different ($P = 0.08$) (Fig. 3). The unusually large standard deviation of the November 1995 0–15-cm FACE-high SOC is a consequence of low SOC of one replicate.

At the end of the second experiment, the only significant differences included 15–30-cm FACE-high with higher SOC than FACE-low ($P = 0.05$), and control-low tending to have more SOC than FACE-low ($P = 0.096$). At 0–15 cm, the seemingly greater FACE-high SOC relative to control-high was not significant ($P = 0.16$). Relative to November 1995, only 0–15-cm FACE-low SOC ($P = 0.04$), and 15–30-cm control-high ($P = 0.04$) and FACE-low ($P = 0.006$) SOC were significantly different (all lower) in May 1997.

Carbon inputs based on isotopic mass balance

To estimate cumulative, 2-yr new carbon inputs in the FACE plots by isotopic mass balance, we used the mixing model:

$$\delta^{13}C_{\text{FACE soil}} = f_{\text{input}}(\delta^{13}C_{\text{input}}) + f_{\text{soil original}}(\delta^{13}C_{\text{soil original}}) \quad \text{Eqn 1}$$

($f_{\text{input}} + f_{\text{soil original}} = 1$; and $\delta^{13}C_{\text{input}} = \delta^{13}C_{\text{plant}}$ are assumed; and $\delta^{13}C_{\text{plant}} = -39.6‰$.) Using $\delta^{13}C_{\text{input}} = -39.6‰$ and $\delta^{13}C_{\text{soil original}}$ values from control plots at the end of the experiment, for the FACE-high and control-high May 1997 pairs, the 0–15, 15–30 and 30–60-cm new carbon fractions are 6.3% ($P = 0.003$ based on mean $\delta^{13}C$ difference), 4.7% ($P = 0.04$) and 4.2% ($P = 0.13$), respectively. For the FACE-low and control-low May 1997 pairs, the 0–15, 15–30 and 30–60-cm fractions are 2.1% ($P = 0.34$), 0.3% ($P = 0.79$) and 3.5% ($P = 0.03$), respectively.

On the other hand, if each treatment's respective November 1995 mean is used as $\delta^{13}C_{\text{soil original}}$ for the treatment, the carbon inputs after the second season for 0–15-cm FACE-high and FACE-low are 3.4% ($P = 0.13$) and 1.0% ($P = 0.32$), respectively. In principle, this also applies to control plots where new carbon can be calculated with the control SOC $\delta^{13}C$ means from November 1995 and the mean control wheat $\delta^{13}C$ of the two seasons (–27.8‰). The May 1997 control-high and control-low values were likewise not significantly different from their respective November 1995 SOC $\delta^{13}C$ counterparts ($P = 0.17$ and $P = 0.37$, respectively), implying no fraction of new carbon. Therefore, we cannot infer with these results any net SOC inputs of new carbon in FACE over control conditions because no differences are significant. There have been previously reported successes with estimating new carbon inputs in control plots without added tracers, however. Nitschelm *et al.* (1997) estimated 10% control plot new carbon inputs after 2 yr in a white clover (*Trifolium repens* L.) FACE experiment with an initial soil-plant $\delta^{13}C$ difference of *c.* 5–6‰, the latter difference being similar in magnitude to the initial conditions of the wheat control plots. After 4 yr, the input of new carbon to controls

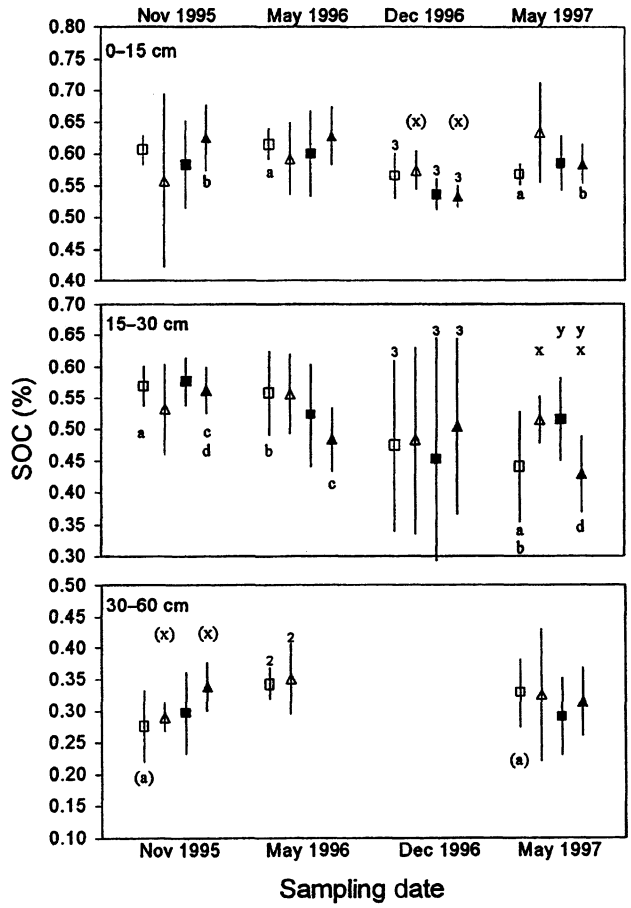

Fig. 3 Mean soil organic carbon (SOC) content of four replicates of all treatments measured throughout the 2-yr experiment at 0–15 cm, 15–30 cm and 30–60 cm. Vertical bars are ±1 SD, and if fewer than four replicates in mean, the actual number (2 or 3) of replicates is placed at the end of the vertical bar. Letters above vertical bars indicate t-test statistically different means among treatment for a given date, and letters below bars indicate repeated measure ANOVA significantly different means of the same treatment on different dates. Control, high N (open squares); FACE, high N (open triangles); control, low N (closed squares); FACE, low N (closed triangles).

of clover and *Lolium perenne* L. in their FACE experiment was about 40%.

Using November 1995 values as $\delta^{13}C_{soil\ original}$ at 15–30 cm and 30–60 cm is problematic because of increasing SOC $\delta^{13}C$ values in some of the treatments over 2 yr (Fig. 2), suggesting only losses of ^{13}C-depleted carbon and no gains. The small FACE-high mean $\delta^{13}C$ declines at 15–30 cm and 30–60 cm over the interval indicating 1.5% and 2.6% new carbon fractions, respectively, are not significant ($P = 0.38$ and $P = 0.44$, respectively).

Although SOC content itself is considered less sensitive than isotopes to small C changes, their record (Fig. 3) might contain corroborating evidence for some isotope outcomes. For example, the SOC content in May 1997 FACE-high appeared to be 10.5% higher than control-high at 0–15 cm, but the difference is not significant ($P = 0.16$). Anomalously, the May 1997 FACE-low SOC tended to be 17.3% less than control-low at 15–30 cm ($P = 0.096$). Relative to the original respective November 1995 SOC 'starting' values, significant SOC content changes include a 7.9% decline ($P = 0.04$) in FACE-low at 0–15 cm in May 1997, a 22.8% drop ($P = 0.04$) in control-high and 23.2% drop ($P = 0.006$) in FACE-low at 15–30 cm, and tendency for a 17.9% increase ($P = 0.07$) in control-high at 30–60 cm. Of these, only the 10.5% SOC increase (not significant) in FACE-high relative to control-high in May 1997 seems to confirm isotopic results, namely the 6.3% new carbon fraction calculated from isotopes. Such an increase is similar in magnitude to the 5% calculated from the 2 yr of FACE wheat $CO_2 \times H_2O$ experiments for the high-H_2O (with high-N) plots (Leavitt *et al.*, 1996).

A 6.3% increase at 0–15 cm and a 4.7% increase estimated from the initial isotope calculations translate to an increase of 0.04% and 0.03% SOC content at 0–15 and 15–30 cm over 2 yr, respectively. These potential increases are generally equal to or less than one standard deviation associated with mean SOC contents, therefore making it less likely that the increase could be discernible from SOC content measurements alone. Furthermore, if SOC is not increasing as new C is added, the new carbon may be simply exchanging with older C, and the SOC contents would not be expected to show a change. Taking the 6.3% and 4.7% isotope-estimated increases over 2 yr and a bulk density of 1.48 g cm^{-3} for the soils in this field (Post *et al.*, 1988), this corresponds to increases in SOC of 41 and 29 g C m^{-2} yr^{-1} at 0–15 and 15–30 cm, respectively, under high-N.

At low-N, mean isotopic differences between FACE and control are small and not significant, suggesting no net carbon additions under CO_2 enrichment at low-N. The effect of limiting N on SOC changes at high CO_2 may be closely linked to plant growth, but the effect of high CO_2-low-N on biomass is not yet fully revealed. For example, Norby *et al.* (1986) working with *Quercus alba* seedlings found enhanced growth at low N levels under high CO_2, although the tissue had a lower N concentrations. In experiments with *Populus grandidentata* under nutrient-limiting conditions, Zak *et al.* (1993) found enhanced growth under high-N and a greater partitioning of biomass to roots. This circumstance might favor conditions for net C additions to the soil. However, Conroy (1992) concluded that CO_2 effects on productivity are greatest under high N availability, and Idso & Idso (1997) report that CO_2 effects on plant growth under limiting N seem to increase as CO_2 enrichment increases. In a FACE experiment with ryegrass and clover, using isotopic tracers Van Kessel *et al.* (2000a, 2000b) found no significant increase in SOC under high CO_2 and ample or low-N.

There is no evidence that different levels of N in the low-N treatment in the two experiments had any effect on these results. The 'trajectories' of apparent isotopic changes (see Soil isotopes in native soil above) seem to have been experienced by all treatments, including high-N treatments that were nearly identical across both years. Furthermore, Sinclair *et al.* (2000) found N-content of leaves in the low-N treatments similarly significantly diminished relative to the high-N treatments in both years. Additionally, the isotope and SOC content results from the single control low-N replicate that accidentally received nine times as much N as the other low-N replicates (but still about 2.5 times lower than the high-N treatment) were within the range of the other three replicates.

C_4 soil subplots for FACE and control isotopic tracing

The $\delta^{13}C$ and SOC for the Randolph and Texas C_4-derived soils are presented in Table 2 as the means (and standard deviations) of the two replicates in which they were positioned. At the beginning of the experiment there were no significant differences of $\delta^{13}C$ and SOC content between FACE and corresponding control values, except in Randolph SOC content. At the end of the second year (May 1997) only Randolph FACE and control $\delta^{13}C$ values were significantly different ($P = 0.02$), with FACE ^{13}C-depleted by 0.56‰. However, Randolph FACE $\delta^{13}C$ mean was unchanged from its November 1995 value, while the control $\delta^{13}C$ mean declined 0.57‰ ^{13}C-enriched relative to its original value, although it is not significant ($P = 0.15$). The Randolph $\delta^{13}C$ results suggest no measurable fraction of new carbon under FACE or under control, although the control plot may have even lost old ^{13}C-depleted carbon. Comparisons of Randolph SOC, however, indicate a significant increase in control SOC ($P = 0.01$) and a decrease in FACE SOC ($P < 0.0001$) between November 1995 and May 1997. The cultivation of these soils might have promoted SOC loss especially in a new plant-climate system in which they were not in equilibrium. Under FACE treatment the loss of only old ^{13}C-enriched SOC would result in lower SOC content but unchanged $\delta^{13}C$. An increase in both Randolph control SOC and $\delta^{13}C$, however, would not be consistent with new wheat C_3 carbon added from the experiment. Decomposition inputs from pre-experiment C_4 plant residues to SOC pools could explain this change.

Table 2 Comparisons of means and standard deviations of soil organic carbon (SOC) $\delta^{13}C$ (‰) and SOC content(%) of 'C_4' subplots in Replicates 1 and 2 (high N) before and after the first and second wheat FACE experiments

		12/95	5/96	5/97
$\delta^{13}C$ (‰)				
Randolph	Blower	−20.34	−20.65	−19.77 x*
		0.29	0.76	0.10
	FACE	−20.30	−20.79	−20.33 x*
		0.14	0.27	0.03
Texas	Blower	−17.41	−18.08	−16.60
		0.86	0.50	0.16
	FACE	−17.41	−17.77	−17.10
		0.86	0.75	0.30
SOC (%)				
		a*		a*
Randolph	Blower	0.75 y*	0.97	0.89
		0.11	0.04	0.11
		b*		b*
	FACE	1.08 y*	0.98	0.85
		0.01	0.12	0.01
		c		c
Texas	Blower	2.03	1.57	1.40
		0.15	0.32	0.01
		d		d
	FACE	2.06	1.44	1.42
		0.19	0.12	0.10

Repeated measures ANOVA comparison on same treatment through time (horizontally, values with same letters above means are different), and one-way ANOVA between FACE and Control ambient for each date (vertically, values with same letters to right of means are different). *F*-test significantly different values are indicated with letter alone ($P < 0.10$) or letters* ($P < 0.05$).
Two subsamples from each replicate were analyzed.

Table 3 Mean $\delta^{13}C$ and standard deviation of wheat plants in ^{13}C-enriched control subplots, and comparisons of means and standard deviations of $\delta^{13}C$ and SOC of soils in Replicates 1 and 2

Tracer wheat plants (4 plants/subplot)		Rep. 1 subplot	Rep. 2 subplot	
	1996	−17.30‰	−13.14‰	
		2.63‰	1.90	
	1997	−12.06	−13.66	
		5.07	4.27	
Soils (mean of reps. 1 & 2)		Depth 0–15	15–30	
$\delta^{13}C$	11/95	−23.00‰	−22.58	a
		0.26‰	0.08	
	5/96	−23.20	−22.18	b
		0.20	0.19	
	5/97	−22.62	−22.06	a,b
		0.08	0.17	
SOC	11/95	0.61%	0.55	c*
		0.04%	0.01	
	5/96	0.59	0.48	c*
		0.06	0.01	
	5/97	0.60	0.51	
		0.08	0.09	

Repeated measures ANOVA comparison on same depth through time (values vertically with same letter designation are different). *F*-test significantly different samples are indicated with letters ($P < 0.1$) and * ($P < 0.05$). Four plants were analyzed from each subplot. Five soil subsamples were analyzed from each subplot replicate in 1996 and 1997. Two soil subsamples were analyzed from each replicate in 1995.

The mean $\delta^{13}C$ of Texas soil subplots in May 1997 was ^{13}C-depleted by *c.* 0.5‰ in FACE relative to the control but not significantly so ($P = 0.17$). As with the Randolph results, the May 1997 FACE $\delta^{13}C$ was close to the original November 1995 value ($P = 0.58$), and the mean May 1997 control $\delta^{13}C$ mean was greater than November 1995 but still not significant ($P = 0.46$). Both FACE and control SOC content tended to be lower at the end of the experiment by about 0.6% ($P = 0.06$ and $P = -0.099$, respectively). These losses of original carbon in both C_4 soils have added complexity in interpretations of results. Isotopic results do not seem to indicate any substantial input of a new carbon fraction under either FACE or control, but transformation of old carbon may have been promoted.

The poor performance of the C_4 subplots is in contrast to the success reported by Ineson *et al.* (1996), who grew birch (*Betula pendula* Roth.) seedlings in pots containing C_4 soils under ambient and elevated CO_2 in a FACE experiment. The soils had been developed under maize for 30 years, acquiring a C_4 signal about 5–7‰ ^{13}C-enriched relative to the birch seedlings. New carbon was seen in the soils under both ambient and elevated CO_2, with an increase of 300% under elevated CO_2. Perhaps the use of soil in pots did not induce as much disturbance as there seemed to be in the wheat FACE C_4 soils evidenced in their carbon losses. Also, removal and drying of the C_4 soils between FACE experiments eliminated the 6–7 months of potential microbial action experienced by soils remaining in the field between seasons.

$^{13}CO_2$ tracing in control plots

Analysis of plants from the $^{13}CO_2$ tracer subplot locations (Table 3) indicates successful bulk ^{13}C enrichment, with means 10–16‰ greater than the adjacent ambient wheat plants in the high-N control plots. The high standard deviations associated with these values are probably a function of chamber differences, heterogeneity in chamber mixing, and the intermittent pulsing of the tracer resulting in isotopically heterogeneous individual plants even when their final bulk composition is as designed.

In May 1997, the $\delta^{13}C$ at both 0–15 cm and 15–30 cm had higher mean values relative to November 1995 ($P = 0.21$ and 0.08, respectively) (Table 3). The 0.52‰ increase at 15–30 cm is consistent with a 6% new carbon fraction assuming

the average wheat $\delta^{13}C$ was −14.0‰. The 0–15 cm difference between November 1995 and May 1997 $\delta^{13}C$ means would translate to a 4.2% new carbon fraction after two growing seasons. The 4–6% new carbon fraction in control plots subjected to pulse labeling is similar to the new C inputs calculated from the commercial gas tracer in FACE plots at 0–15 cm, but less significant. If the pulse-labeled subplots are representative of activity in full control plots, this suggests there is no net increase in new carbon fraction in FACE relative to ambient conditions. Although there was no apparent increase in SOC content at either depth in pulse-labeled subplots or in the larger field, 6% added carbon would raise SOC content from about 0.60% to 0.64%, a change that is generally within the precision associated with mean SOC contents.

This is the first study, of which we are aware, that has used a pulsed isotope tracer to attempt to quantify SOC inputs in control treatments, although other elevated-CO_2 studies have used isotopic tracers in control conditions for other purposes. Hungate *et al.* (1997) pulsed ^{13}C-enriched CO_2 tracer in an open-top tracer study of natural grasslands, but its purpose was to help apportion soil respiration between root and heterotrophic sources. Van Vuuren *et al.* (2000) exposed wheat to ^{13}C-depleted CO_2 under both ambient and elevated CO_2 concentrations in a growth-chamber study, producing ^{13}C-depleted roots (−38 to −41‰) to quantify root- vs soil-derived CO_2. Over the 116 d of the experiment, this strongly ^{13}C-depleted root matter did not produce a detectable signal in the soil carbon.

Summary and Conclusions

The use of a highly ^{13}C-depleted commercial CO_2 gas for elevating atmospheric CO_2 in FACE plots produced FACE wheat plants that were 11–12‰ ^{13}C-depleted relative to ambient plants. There was no isotopic difference between high-N and low-N wheat plants in FACE or control CO_2 plots. These FACE plants were in turn 16–18‰ ^{13}C-depleted relative to resident soil organic carbon, providing a good opportunity to quantitatively determine the amount of new carbon entering soil pools, just as had been done previously with FACE cotton and wheat $CO_2 \times H_2O$ experiments (Leavitt *et al.*, 1994, 1996).

Except for the 30–60-cm soil samples, SOC content and $\delta^{13}C$ were fairly uniform among all treatment positions prior to the first experiment. There is evidence of some synchronous shifting of SOC content and $\delta^{13}C$ during the sequence of experiments, possibly related to differential contributions (and losses) from residues of a prior crop and wheat input from the experiment. Sampling only at the beginning of the first experiment and at the end of the second would not have revealed this subtle evolution.

The strongest demonstration of C inputs is seen in the high-N, 0–15-cm FACE-control isotopic difference that initially manifests itself at the beginning of the second experiment and persists to the end. This 6.3% new C fraction corresponds to turnover of 3.2% yr^{-1} or *c.* 41 g C m^{-2} yr^{-1}. This new C fraction is similar to that observed in a previous FACE wheat experiment under similar experimental conditions. A 4.7% new carbon fraction at 15–30 cm under high-N is also significant (*c.* 29 g C m^{-2} yr^{-1}).

The need to attain a stronger isotopic tracer under control conditions prompted two ancillary experiments. ^{13}C-enriched exotic soils developed under C_4 vegetation were installed as subplots in high-N FACE and control plots of replicates 1 and 2. Under most experimental conditions, they lost a significant amount of SOC, possibly because of cultivation and the new agrosystem into which they were introduced. In fact, the trends in SOC $\delta^{13}C$ suggest no new carbon was added to drive the SOC more ^{13}C-depleted.

Enriched $^{13}CO_2$ was added as a pulse label to subplots in control-high(N) replicates 1 and 2 plots to provide a strong tracer in the wheat plants, 14‰ ^{13}C-enriched relative to ambient plants and 8–9‰ more ^{13}C-enriched than that of the local SOC. There is evidence this signal was not homogeneous in the plants, but nonetheless, $\delta^{13}C$ tends to increase from beginning to end of the experiment at 15–30 cm, consistent with 6% new carbon fraction at the end of the experiment. This implies a turnover rate of about 3% yr^{-1}. There was no change in SOC content over the same period. The 6% new carbon fraction for controls is nearly identical to the input measured in 0–15-cm FACE plots, suggesting the elevated CO_2 treatment did not provide a measurable change in the soil carbon turnover. FACE experiments with effective isotopic tracers uniformly and continuously operating throughout all treatments would eliminate ambiguities in trying to assess dynamics under FACE and control conditions. Finally, there remains the possibility that future decomposition of plant detritus (that we have intentionally removed in processing) may produce FACE-control differences that are not present at the end of the 2-yr FACE experiment.

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References

Boutton TW. 1991. Stable carbon isotope ratios of natural materials: I. Sample preparation and mass spectrometric analysis. In: Coleman DC, Fry B, eds. *Carbon Isotope Techniques*, San Diego, CA, USA: Academic Press, 173–175.

Brooks TJ, Wall GW, Pinter PJ Jr, Kimball BA, LaMorte RL, Leavitt SW, Matthias AD, Adamsen FJ, Hunsaker DJ, Webber AN. 2001. Acclimation response of spring wheat in a free-air CO_2 enrichment (FACE) atmosphere with variable soil nitrogen regimes. 3. Canopy architecture and gas exchange. *Photosynthesis Research* (In press).

Cambardella CA, Elliott ET. 1992. Particulate soil organic-matter changes across a grassland cultivation sequence. *Soil Science Society of America Journal* **56**: 777–783.

Canadell JP, Pitelka LF, Ingram SI. 1996. The effects of elevated [CO_2] on plant-soil carbon below-ground: a summary and synthesis. *Plant and Soil* **187**: 391–400.

Conroy JP. 1992. Influence of elevated atmospheric CO_2 concentrations on plant nutrition. *Australian Journal of Botany* **40**: 445–456.

Coplen TB. 1995. Discontinuance of SMOW and PDB. *Nature* **375**: 285.

Craig H. 1957. Isotopic standards for carbon and oxygen and correction factors for mass-spectrometric analysis of CO_2. *Geochimica et Cosmochimica Acta* **12**: 133–149.

Curtis PS, O'Neill EG, Teeri JA, Zak DR, Pregitzer KS. 1994. Below ground responses to rising atmospheric CO_2: Implications for plants, soil biota and ecosystem processes. *Plant and Soil* **165**: 1–6.

Dugas WA, Pinter Jr PJ. 1994. The free-air carbon dioxide enrichment (FACE) cotton project: a new field approach to assess the biological consequences of global change. *Agricultural and Forest Meteorology* **70**: 1–342.

Dzurec RS, Boutton TW, Caldwell MM, Smith BN. 1985. Carbon isotope ratios of soil organic matter and their use in assessing community composition changes in Curlew Valley, Utah. *Oecologia* **66**: 17–24.

Garcia RL, Long SP, Osborne CP, Kimball BA, Nie GY, Pinter Jr PJ, LaMorte RL. 1998. Photosynthesis and conductance of spring-wheat leaves: Field response to free-air CO_2 enrichment. *Plant, Cell & Environment* **21**: 659–669.

Hendrey GR, ed. 1992. FACE: Free-Air CO_2 Enrichment for Plant Research. *Critical Review of Plant Sciences* **11**: 1–308.

Hungate BA, Holland EA, Jackson RB, Chapin FS III, Mooney HA, Field CB. 1997. The fate of carbon under carbon dioxide enrichment. *Nature* **388**: 576–579.

Idso KE, Idso SB. 1997. A synopsis of a major review of plant responses to rising levels of atmospheric carbon dioxide in the presence of unfavorable growing conditions. In: Allen Jr LH, Kirkham MB, Olszyk DM, Whitman CE, eds. *Advances in carbon dioxide effect research*. Madison, WI, USA: American Society of Agronomy Special Publication no. 61, 131–139.

Ineson P, Cortufo MF, Bol R, Harkness DD, Blum H. 1996. Quantification of soil carbon inputs under elevated CO_2: C_3 plants in a C_4 soil. *Plant and Soil* **187**: 345–350.

Keeling CD, Mook WG, Tans PP. 1979. Recent trends in $^{13}C/^{12}C$ ratio of atmospheric carbon dioxide. *Nature* **277**: 121–123.

Kimball BA, Pinter Jr PJ, Garcia RL, LaMorte RL, Wall GW, Hunsaker DJ, Wechsung G, Wechsung F, Kartschall Th. 1995. Productivity and water use of wheat under free-air CO_2 enrichment. *Global Change Biology* **1**: 429–442.

Kimball BA, LaMorte RL, Pinter Jr PJ, Wall GW, Hunsaker DJ, Adamsen FJ, Leavitt SW, Thompson TL, Matthias AD, Brooks TJ. 1999. Free-air CO_2 enrichment (FACE) and soil nitrogen effects on energy balance and evapotranspiration of wheat. *Water Resources Research* **35**: 1179–1190.

Leavitt SW, Paul EA, Galadima A, Nakayama FS, Danzer SR, Johnson H, Kimball BA. 1996. Carbon isotopes and carbon turnover in cotton and wheat FACE experiments. *Plant and Soil* **187**: 147–155.

Leavitt SW, Paul EA, Kimball BA, Hendrey GR, Mauney JR, Rauschkolb R, Rogers H Jr, Lewin KF, Nagy J, Pinter Jr PJ, Johnson HB. 1994. Carbon isotope dynamics of CO_2-enriched FACE cotton and soils. *Agricultural and Forest Meteorology* **70**: 87–101.

Leavitt SW, Paul EA, Pendall E, Pinter Jr PJ, Kimball BA. 1997. Field variability of carbon isotopes in soil organic carbon. *Nuclear Instrumentation and Methods in Physics Research* **123**: 451–454.

Martens DA. 2000. Plant residue biochemistry regulates soil carbon cycling and carbon sequestration. *Soil Biology & Biochemistry* **32**: 361–369.

Nitschelm JJ, Luscher A, Hartwig UA, Van Kessel C. 1997. Using stable isotopes to determine soil carbon input differences under ambient and elevated atmospheric CO_2 conditions. *Global Change Biology* **3**: 411–416.

Norby RJ, Pastor J, Melillo JM. 1986. Carbon–nitrogen interactions in CO_2-enriched white oak: Physiological and long-term perspectives. *Tree Physiology* **2**: 233–241.

Pinter Jr PJ, Kimball BA, Wall GW, LaMorte RL, Hunsaker DJ, Adamsen FJ, Frumau KFA, Vugts HF, Hendrey GR, Lewin KF, Nagy J, Johnson JB, Wechsung F, Leavitt SW, Thompson TL, Matthias AD, Brooks TJ. 2000. Free-air CO_2 enrichment (FACE): blower effects on wheat canopy microclimate and plant development. *Agricultural and Forest Meteorology* **103/4**: 319–332.

Post DF, Mack C, Camp PD, Suliman AS. 1988. Mapping and characterization of the soils on the University of Arizona Maricopa Agricultural Center. In: *Hydrology and water resources of the Southwest.* Tuscon, AZ, USA: Arizona-Nevada Academy of Science **18**: 49–60.

Power JF, Doran JW, Wilhelm WW. 1986. Uptake of nitrogen from soil, fertilizer, and crop residues by no-till corn and soybean. *Soil Science Society of America Journal* **50**: 137–142

Sinclair TR, Pinter Jr PJ, Kimball BA, Adamsen FJ, LaMorte RL, Wall GW, Hunsaker, DJ, Adam N, Brooks TJ, Garcia RL, Thompson T, Leavitt S, Matthias A. 2000. Leaf nitrogen concentration of wheat subjected to elevated [CO_2] and either water or nitrogen deficits. *Agriculture, Ecosystems, and Environment* **79**: 53–60.

Torbert HA, Rogers HH, Prior SA, Schlesinger WH, Runion GB. 1997. Effects of elevated atmospheric CO_2 in agro-ecosystems on soil carbon storage. *Global Change Biology* **3**: 513–521.

Van Kessel C, Nitschelm J, Horwath WR, Harris D, Walley F, Lüscher A, Hartwig U, Luscher A. 2000a. Carbon-13 input and turn-over in a pasture soil exposed to long-term elevated atmospheric CO_2. *Global Change Biology* **6**: 123–135.

Van Kessel C, Horwath WR, Hartwig U, Harris D, Lüscher A. 2000b. Net soil carbon input under ambient and elevated CO_2 concentrations: Isotopic evidence after 4 years. *Global Change Biology* **6**: 435–444.

Van Vuuren MMI, Robinson D, Scrimgeour CM, Raven JA, Fitter AH. 2000. Decomposition of ^{13}C-labelled wheat root systems following growth at different CO_2 concentrations. *Soil Biology & Biochemistry* **32**: 403–413.

Wedin DA, Tieszen LJ, Dewey B, Pastor J. 1995. Carbon isotope dynamics during grass decomposition, soil organic matter formation. *Ecology* **76**: 1383–1392.

Zak DR, Pregitzer KS, Curtis PS, Teeri JA, Fogel R, Randlett DL. 1993. Elevated atmospheric CO_2 and feedback between carbon and nitrogen cycles. *Plant and Soil* **151**: 105–117.