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Dynamics of labile and recalcitrant soil carbon pools in a sorghum free-air CO_2 enrichment (FACE) agroecosystem

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Abstract

Experimentation with dynamics of soil carbon pools as affected by elevated CO_2 can better define the ability of terrestrial ecosystems to sequester global carbon. In the present study, 6 N HCl hydrolysis and stable-carbon isotopic analysis ($\delta^{13}C$) were used to investigate labile and recalcitrant soil carbon pools and the translocation among these pools of sorghum residues isotopically labeled in the 1998–1999 Arizona Maricopa free air CO_2 enrichment (FACE) experiment, in which elevated CO_2 (FACE: 560 μmol mol^{-1}) and ambient CO_2 (Control: 360 μmol mol^{-1}) interact with water-adequate (wet) and water-deficient (dry) treatments. We found that on average 53% of the final soil organic carbon (SOC) in the FACE plot was in the recalcitrant carbon pool and 47% in the labile pool, whereas in the Control plot 46% and 54% of carbon were in recalcitrant and labile pools, respectively, indicating that elevated CO_2 transferred more SOC into the slow-decay carbon pool. Also, isotopic mixing models revealed that increased new sorghum residue input to the recalcitrant pool mainly accounts for this change, especially for the upper soil horizon (0–30 cm) where new carbon in recalcitrant soil pools of FACE wet and dry treatments was 1.7 and 2.8 times as large as that in respective Control recalcitrant pools. Similarly, old C in the recalcitrant pool under elevated CO_2 was higher than that under ambient CO_2, indicating that elevated CO_2 reduces the decay of the old C in recalcitrant pool. Mean residence time (MRT) of bulk soil carbon at the depth of 0–30 cm was significantly longer in FACE plot than Control plot by the averages of 12 and 13 yr under the dry and wet conditions, respectively. The MRT was positively correlated to the ratio of carbon content in the recalcitrant pool to total SOC and negatively correlated to the ratio of carbon content in the labile pool to total SOC. Influence of water alone on the bulk SOC or the labile and recalcitrant pools was not significant. However, water stress interacting with CO_2 enhanced the shift of the carbon from labile pool to recalcitrant pool. Our results imply that terrestrial agroecosystems may play a critical role in sequestrating atmospheric CO_2 and mitigating harmful CO_2 under future atmospheric conditions.
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Keywords: Elevated CO_2; $\delta^{13}C$; Soil recalcitrant and labile carbon pools; SOC dynamics; Mean residence time; Sorghum FACE

1. Introduction

Elevated carbon dioxide (CO_2) is well known to increase the total biomass of terrestrial ecosystems by stimulating plant photosynthesis, but possible effects on soil organic carbon (SOC) storage are still intensively debated because of the uncertainty of the SOC dynamics under elevated CO_2 and because of the short-term nature of most CO_2-enrichment experiments. Soil is the biggest carbon (C) pool in the Earth's terrestrial ecosystem, containing 1500–1600 Pg C in organic matter, which is about 3 and 4 times as large as the C content in the atmosphere and terrestrial biosphere, respectively (Lal et al., 1997;

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Stevenson and Cole, 1999; Lal, 2004). Given the size of the soil C pool, a very slight increase in soil C content could cause meaningful sequestration of excess atmospheric CO_2. However, detecting the change in total SOC content is very difficult, simply because soil is such a large C reservoir and the changes of parameters in question are so small, especially for short-term experiments (Hungate et al., 1996). Presently, total soil C content is determined with an accuracy of $\pm 2\%$ of the total amount present, which means that management effects cannot usually be fully evaluated until decades after treatments are initiated (Paul et al., 2001a). This has limited our understanding of behavior of soils as C sinks or sources under increased atmospheric CO_2.

Determination of CO_2 effects on soil carbon processes requires more accurate understanding of soil carbon sub-pool dynamics. Soil organic matter consists of a spectrum of materials ranging in mean residence time (MRT) from less than a few weeks for plant residues and root exudates to greater than several thousand years for the resistant humic substances. Therefore, SOC can be fractionated into several different pools with various MRTs. Each carbon pool plays a very different role in SOC dynamics and soil carbon sequestration. If a plant–soil system encourages the translocation of soil organic matter into labile pools with short-MRT under elevated CO_2, microbial decomposition will be rapid and consequently decrease soil carbon storage. In contrast, if elevated CO_2 promotes more soil organic matter entering recalcitrant pools with long-MRT, SOC stability and storage will increase, and have the greatest long-term impact on C sequestration. Thus, knowledge of the distribution of SOC in different kinetic pools is essential for understanding the dynamics of the SOC under given environmental conditions such as elevated CO_2 (Trumbore, 1993).

To separate and characterize soil carbon pools, researchers have used various physical, chemical and biological methods, including particle size and density fractionation (Cambardella and Elliott, 1992; Trumbore, 1993; Jastrow et al., 1996), humic fractionation (Campbell et al., 1967; Nissenbaum and Schallinger, 1974), molecular fractionation (Sorensen and Paul, 1971; Johnson, 1986; Martens and Frankenberger, 1991), and conceptual fractionation with models (Parton et al., 1987; Hsieh, 1989). Unfortunately, none of those fractionation methods can satisfactorily and practically separate soil carbon pools on the basis of stability. For example, particle size and density fractionations have been shown to yield pools of SOC with different properties regarding chemical composition and turnover (Tiessen and Stewart, 1983; Christensen, 2001; Roscoe et al., 2001). The MRTs associated with the particle sizes, however, had little correlation with SOC decomposition rate in the field soil profile (Christensen, 1992; Balesdent, 1996; Jastrow et al., 1996; Roscoe et al., 2001). Humic fractionation was one of the traditional methods that separated total SOC into humic fractions. Although humic acid and humin fractions were usually older (1130–1410 yr) than the corresponding fulvic acid fraction (50–550 yr), Campbell et al. (1967) found that a portion of humic acid and humin substance were still active with a short MRT (25–465 yr). This indicates the incomplete separation of labile from recalcitrant pool with humic fractionation. Models of soil C dynamics usually separate SOC into 2–5 pools. For example, Parton et al. (1987) simulated SOC levels in Great Plains grasslands using a model to separate soil C pools with various MRTs into a metabolic pool (0.1–1 yr), a plant structural pool (1–5 yr), an active pool (1–5 yr), a slow pool (20–40 yr), and a passive pool (200–1500 yr). The problem with modeling methods in many cases is their inability to represent a real soil situation as they lack analytical information on the physical and chemical properties of the SOC. Molecular fractionation divides soil organic matter into specific chemical compounds. The newly formed C and old SOC in those compounds, however, are not necessarily easy to date and separate (Johnson, 1986; Martens and Frankenberger, 1991).

Regardless of differences among methods, the simplest fractionation may be to divide SOC into two major pools: a labile pool characterized by an MRT of years to a few decades, which is affected by variations of environmental factors over short periods, and a recalcitrant pool with MRTs ranging from hundreds to thousands of years, which is particularly relevant to the role of soil as a long-term terrestrial C sink in the global carbon cycle. Hence, differentiating the recalcitrant from the labile C pool in soil systems is important to the study of SOC dynamics under elevated CO_2. Currently, most research into the effect of CO_2 on soil C pool are focused on the change in total SOC rather than soil carbon sub-pools. In the present study, we used 6 N HCl hydrolysis exploited by Campbell et al. (1967) and Leavitt et al. (1996) to separate SOC into two pools, taking the residue fraction after the hydrolysis as a recalcitrant pool and the hydrolyzate (or supernatant) fraction as a labile pool.

The fractionation with 6 N HCl hydrolysis is the simplest and most reproducible method for not only separating young from old organic carbon but also giving meaningful separation of easily degraded and resistant carbon pools. Researchers have proven that the hydrolyzates after 6 N HCl hydrolysis were much younger than bulk SOC and the residues much older than bulk SOC (Campbell et al., 1967; Leavitt et al., 1996; Paul et al., 2001b, 2006), indicating that the hydrolyzable organic carbon fraction is more active and non-hydrolysable organic C represents a more stable fraction of SOC. Leavitt et al. (1996) analyzed 65 soil samples collected from Michigan, Arizona and the Great Plains with 6N HCl and then used radiocarbon (^{14}C) to date each fraction. They found that organic carbon in the acid-hydrolysis residue fractions (ranging from 170 ± 60 BP to 4900 ± 50 BP) was much older than the carbon in the hydrolyzate fractions (modern to 340 ± 60 BP). Paul et al. (2001b) reported the MRTs, dated by radiocarbon (^{14}C), of non-hydrolyzable C in Midwestern agricultural soils were older than the total C by an average of 1338 years, and the hydrolyzable C fractions were generally several hundreds to thousands of years younger than the total SOC. Collins et al. (2000) used 6N HCl hydrolysis to divide US Corn Belt soil C into a resistant pool (residues of acid hydrolysis) and a hydrolyzate pool. Then, using a curve-fitting model of respired CO_2 released from laboratory incubation, they further divided the hydrolyzate C into active and slow C pools. They reported that the active pool comprised 3–8% of the SOC with an average field MRT of 100 d, and the slow pool comprised 50–65% of SOC with MRTs of 12–28 yr. Compared to the resistant pool, which constituted 30–50% of total SOC with a MRT of 2600 yr, the active and slow pools could both be considered as modern. This implies that the isolation of one labile pools and one recalcitrant pool has the potential to contribute to assessment of both short- and long-term soil C response to changes in the environment, such as rising CO_2 in the atmosphere.

It was reported that, besides humic substances (Campbell et al., 1967), 6 N HCl hydrolysis also leaves behind other resistant compounds derived from new plant residues, such as lignin (Leavitt et al., 1996; Collins et al., 2000) and fat resins and suberins (Rovira and Vallejo, 2002). All of these compounds are highly resistant to chemical and biological degradation (Minderman, 1968). Such easily degradable compounds as amino acids, amino sugar, soluble carbohydrates, and components of the microbial biomass (Hu et al., 1997; Hobbie et al., 2002; Rovira and Vallejo, 2002) are major constituents of the acid-hydrolyzable fraction. This further indicates that the 6 N HCl hydrolysis method effectively separates labile carbon from recalcitrant pool, as we would expect. It should be pointed out that some labile carbon compounds chemically separated by 6N HCl hydrolysis may be protected by soil matrix and minerals or by the encapsulation in refractory and macromolecular organic matter against biological degradation (Knicker and Hatcher, 1997; Baldock and Skjemstad, 2000; Zang et al., 2001).

Stable carbon isotopic techniques (based on $^{13}C/^{12}C$) provide a highly accurate method to trace carbon transfer among the pools, and have been successfully used in studies of SOC dynamics (Martel and Paul, 1974; Nissenbaum and Schallinger, 1974; Anderson and Paul, 1984; Tieszen and Boutton, 1989; Cerling et al., 1991; Hsieh, 1992; Leavitt et al., 1994, 1996, 1997; Jastrow et al., 1996; Paul et al., 2001b; Stevenson et al., 2005). The isotopic composition of soil organic C reflects the plant materials from which it is derived. If a soil developed primarily in association with C_3 plants was then planted with C_4 vegetation, the $^{13}C/^{12}C$ ratio of SOC would increase from contributions of organic matter from C_4 plant. This is because plants with the C_3 photosynthetic pathway strongly discriminate against $^{13}CO_2$ during photosynthesis, causing the $^{13}C/^{12}C$ ratio of their phytomass to be depleted in ^{13}C relative to those of C_4 plants that do not discriminate as much against $^{13}CO_2$. Therefore, the introduction of a crop with a different photosynthetic pathway provides an isotopic label of new input C.

The present study was conducted at Maricopa, Arizona, free-air CO_2 enrichment (FACE) experiment in which C_4 sorghum was grown on a field previously cultivated primarily with C_3 crops (cotton and wheat), and the average $\delta^{13}C$ value of SOC was about $-22.68‰$. The newly input sorghum residues were highly enriched in ^{13}C compared to original SOC, and have $\delta^{13}C$ values ranged from $-10.27‰$ to $-11.68‰$. The large difference in the $\delta^{13}C$ values of organic sources enables us to trace the translocation of new and old SOC among soil C pools under both ambient and elevated CO_2. The objectives of this study were to (1) separate soil labile and recalcitrant pools by using 6 N HCl hydrolysis, (2) determine the transfer of new C_4-sorghum residues among these pools using isotope techniques, and (3) evaluate the impact of elevated CO_2 on the soil labile and recalcitrant pools associated with the change in the MRT of bulk soil C under adequate and deficient water supply conditions. This study was not focused on the effects of elevated CO_2 and water on total bulk SOC in the FACE experiment, which have been addressed by Leavitt et al (in revision).

2. Materials and methods

2.1. FACE site description

The sorghum [*Sorghum bicolor* (L.) Möench] FACE experiment was conducted continuously for two growing seasons at the University of Arizona Maricopa Agricultural Farm (elevation: 358 m, 33.1 °N, 112.0 °W), AZ, USA, in 1998 and 1999. Detailed methods have been presented by Ottman et al. (2001), Conley et al. (2001) and Wall et al. (2001). Briefly, the FACE experiment consisted of eight 25-m-diameter rings, randomly distributed in a 12-ha sorghum field. All eight rings were equipped with identical computer-regulated blower systems, but only four of them received additional pure tank CO_2 as FACE treatments, and the other four received ambient air as Control treatments. CO_2 fumigation was applied continuously (24 h per day) from the date when 50% of the sorghum plants emerged until plant maturity. Average daytime CO_2 concentrations monitored in the center of each array at 10 cm above the crop canopy during 1998 and 1999 growing seasons were 556 and 566, and 364 and 373 $\mu mol\,mol^{-1}$ in FACE and Control plots, respectively. Average nighttime CO_2 concentrations were slightly higher at 603 and 607, and 428 and 433 $\mu mol\,mol^{-1}$ at FACE and Control plots, respectively. Each of 8 rings, for both FACE and Control, was split into wet and dry sides in semicircular halves—the wet side received 1218 and 1047 mm of irrigation + rain applied during 1998 and 1999 growing seasons, respectively, which was slightly more than twice the amount of water received on the dry side (474 and 491 mm during 1998 and 1999 growing seasons, respectively). Therefore, total treatments in the

FACE experiment were CD (Control CO_2 + dry H_2O), FD (FACE CO_2 + dry H_2O), CW (Control CO_2 + wet H_2O), and FW (FACE CO_2 + wet H_2O).

Soil at the FACE site was classified as a Trix clay loam: fine-loamy mixed (calcareous), hyperthermic Typic Torrifluvent (Post et al., 1988; Kimball et al., 1992), which was formed on a relict basin floor of Pleistocene age and affected by Holocene-age alluvium deposited adjacent to the Santa Cruz Wash. Fine-textured recent alluvium (clay loam) makes up the whole soil from surface to 100 cm with 25–45% sand, 15–48% silt, and 27–40% clay. The subsurface horizon (30–100 cm) has similar characteristics as the surface horizon (0–30 cm). Soil bulk density (SBD) averages 1.218, 1.265, 1.325, 1.385, 1.478, and 1.570 g cm^{-3} at depths of 0–15, 15–30, 30–45, 45–60, 60–80, and 80–100 cm, respectively (the SBD is the mean of two replicates, and was calculated based on the weight of soil collected with known volume of container from FACE and Control replicate 1 and 2, and then oven-dried at 105 °C for more than 24 h to constant weight). Soil organic matter originated from both native vegetation (e.g., CAM and C_4 plants) and frequent cultivation of C_3 plants (e.g., cotton and wheat) since early 1950s as farmland. SOC contents ranges from about 7 g kg^{-1} in the surface horizon to 2 g kg^{-1}down at 100-cm depth. Because surface SOC was mainly derived from recently cultivated C_3-crops and deep SOC was largely formed from native CAM plants, the $\delta^{13}C$ of SOC was more ^{13}C-depleted in the surface layer ($\delta^{13}C = -22.68$‰) than in the deep layer ($\delta^{13}C = -19.21$‰). C_4-sorghum residues in the FACE experiment, however, were even more ^{13}C-enriched ($\delta^{13}C$ ranging from −10.27‰ to −11.69‰). This significant difference in carbon isotopic composition between SOC and sorghum provides a strong isotopic tracer for probing the translocation of the sorghum residues in the soil system.

2.2. *Soil sampling and pretreatment*

Soil samples were augered from each of the FACE and Control plots in July 1998 before CO_2 application and in September 1999 at the end of second growing season. Detailed methods for soil sampling and pretreatment have been given by (Leavitt et al., 1994; Leavitt et al., in revision). Briefly, soils were cored from four locations in each plot with a 5.5-cm-diameter auger down to 60 cm in 1998 and 100 cm in 1999 at quadrant positions 5-6 m from the center of the rings, namely two from wet side and two from dry side. Each hole was sampled at depths of 0–15, 15–30, 30–45, 45–60, 60–80, and 80–100 cm (0–15, 15–30, 30–60 cm for 1998 soil) and pooled by depth increment within moisture regime and plot. The soils from 30–45 to 60–80 cm depths are not analyzed in this paper. After removal of recognizable stone and plant fragments by hand picking, air-dried soil was passed through a 2-mm sieve.

Because soil carbon pools separated with 6 N HCl hydrolysis can be strongly affected by new fresh plant residue (Leavitt et al., 1996), the sieved soil sub-sample was further treated to remove remaining fine plant residue fragments and roots. (In fact, the visible plant residues are considered as a specific carbon pool (Jastrow et al., 1996; Roscoe et al., 2001), which is excluded in this paper.) First, about 20–30 g of the soil sample was immersed in 150 ml of 1 N HCl (to remove carbonate from soil), stirring occasionally and then sitting in the acid overnight to remove soil carbonates. After the acidified soil was filtered with a vacuum filter and rinsed with deionized (DI) water free of HCl, the soil sample was subsequently immersed in 200–300 ml of 1.2 g cm^{-3} NaCl solution to float and skim off fine plant residue fragments. The floating and skimming was repeated until no more plant residues floated to the surface. Then, the soil was filtered, rinsed with DI water free of salt, and dried on a hot plate at 60–70 °C. After dry, the soil sample was ground with a mortar and pestle through a 1-mm sieve, and examined again under a 20 × microscope to remove any remaining identifiable plant fragments.

2.3. *Soil pool separation*

Acid hydrolysis with 6 N HCl was performed on all of the soils that were collected from each soil horizon in 1999 after removal of carbonates and plant fragments. The procedure for this method was modified from Leavitt et al. (1996). Ten grams of soil were placed into a 500 ml round-bottom flask with 150 ml of 6 N HCl. With a water-cooled condenser installed above the flask, the mixture was heated to boiling at 116 °C with an electric heating mantle for ca. 18 h. After hydrolysis, the mixture was allowed to cool down and centrifuged at 1500*g* for 15–20 min. Supernatant liquid was decanted to a preweighed beaker and non-hydrolyzed residue was rinsed with 20 ml DI water. The rinsing solutions were decanted to the beaker containing the supernatant. The process of centrifuging and decanting was repeated twice with DI water to remove all soluble materials from residue. The residue was then recovered by transferring to preweighed vials. Both hydrolyzate (supernatant) fraction, classified as the labile pool, and the resistant (residue) fraction, taken as the recalcitrant pool, were dried on hot plate at 60−70 °C to constant weight, ground through a 1-mm sieve and analyzed for carbon and $\delta^{13}C$.

2.4. *Carbon content and stable-isotope analysis*

Whole soil and soil fraction (labile and recalcitrant pools) were analyzed for total C content and stable carbon isotopic composition ($\delta^{13}C$). The total C and $\delta^{13}C$ were equivalent to those of organic carbon because we had pretreated the soil free of carbonates. Whole soil was analyzed on a Finnigan-MAT Delta S (Finnigan MAT GmbH, Breman/Germany) dual inlet gas source isotope ratio mass spectrometer in 2000, by injecting pure CO_2 prepared off-line into the mass spectrometer. To convert

SOC into pure CO_2, about 100 mg of each pretreated soil was sealed in evacuated quartz tubes with copper oxide and silver foil combusted at 900 °C for 2 h and then switched to 650 °C for another 2 h, according to the procedures described by Boutton (1991). The CO_2 product was then collected and cryogenically purified in a vacuum line. After the carbon yield was manometrically determined, the purified CO_2 was injected in the Finnigan MAT Delta S (Finnigan MAT GmbH, Breman/Germany) mass spectrometer to measure the $^{13}C/^{12}C$ ratio (reported as $\delta^{13}C$). The C% and $^{13}C/^{12}C$ ratio in soil labile and recalcitrant pools were determined in 2004 on a new Finnigan Delta PlusXL (Finnigan MAT GmbH, Breman/Germany) continuous-flow gas-ratio mass spectrometer, which used an elemental analyzer (Costech) to combust soils on-line, purify the byproducts and inject CO_2 into the mass spectrometer. For this mass spectrometer, about 30–50 mg of soil sample was weighed into a small tin capsule that was automatically handled by an elemental analyzer. Analysis in the Finnigan Delta PlusXL mass spectrometer yielded both carbon content and isotopic composition. The precision of both mass spectrometers was ca. ±0.06‰ for repeated analysis of the same organic standard samples.

2.5. Data calculations

The stable carbon isotopic composition was expressed relative to the international standard PDB (the international Vienna Pee Dee Belemnite) as $\delta^{13}C$ in units of permil (‰). The equation describing $\delta^{13}C$ is

$$\delta^{13}\mathrm{C}(‰) = \left(\frac{R_{\mathrm{sample}}}{R_{\mathrm{standard}}} - 1\right) \times 10^3,$$

where, R is the $^{13}C/^{12}C$ ratio of the sample or the standard (PDB: $^{13}C/^{12}C = 0.0112372$). The fraction of soil C derived from the new sorghum residue input (f_{new}) was calculated with the isotopic mixing model (Leavitt et al., 1994) as

$$f_{\mathrm{new}} = \frac{\delta^{13}C_{\mathrm{sample}} - \delta^{13}C_{\mathrm{old}}}{\delta^{13}C_{\mathrm{new}} - \delta^{13}C_{\mathrm{old}}},$$

where, $\delta^{13}C_{\mathrm{sample}}$ and $\delta^{13}C_{\mathrm{old}}$ are the isotopic signatures of SOC after and before the sorghum FACE experiment, respectively. $\delta^{13}C_{\mathrm{new}}$ represents the $\delta^{13}C$ value of newly input C, C_4-sorghum residues. In this calculation, we assumed that no isotopic discrimination occurred during microbial decomposition of SOC and sorghum residues. Cadisch and Giller (2001) reported no change in $\delta^{13}C$ values of decomposing *Brachiaria humidicola* and *Desmodium ovalifolium* residues after 1 year incubation. However, Mary et al (1992) observed a slight decrease in the $\delta^{13}C$ value of maize residues after 50-d decomposition, and Foereid et al. (2004) showed a slight increase in the $\delta^{13}C$ values after 135-d decomposition of grass (*Miscanthus*) root.). The fraction of original (old) SOC was calculated as: $f_{\mathrm{old}} = 1 - f_{\mathrm{new}}$. Here, we assumed all SOC after 2-year FACE experiment had only two sources: remaining original SOC and inputs of new sorghum residues.

The percentages of newly input C (C_{new}%) and original SOC remaining (C_{old}%) in the soil were calculated based on their fraction (f) and total SOC content(C_{total}%) as

$$C_{\mathrm{new}}\% = f_{\mathrm{new}} \times C_{\mathrm{total}}$$

and

$$C_{\mathrm{old}}\% = f_{\mathrm{old}} \times C_{\mathrm{total}}.$$

To determine the decomposition rate and mean residence time (MRT) of SOC, we used the following single exponential decay model:

$$X_t = X_0 e^{-kt},$$

and

$$\mathrm{MRT(yr)} = \frac{1}{k} = -\frac{t(\mathrm{yr})}{\ln(X_t/X_0)},$$

where, X_t is the original SOC remaining after t years; X_0 is the SOC content prior to the CO_2 experiment; k is the decomposition constant; t is the time elapsed since CO_2 fumigation, 1.25 yr used in the present paper. This model assumes (1) bulk soil carbon turnover with only one carbon pool of uniform turnover, and (2) SOC decomposition follows first-order kinetics at steady-state conditions. The decomposition of SOC per unit time was introduced as turnover rate equivalent to the decay rate or decomposition rate (k). Although the rate of decomposition of SOC does not strictly follow first-order kinetics, we used the model for general comparative purposes. The calculation of MRT was only applied on bulk SOC because we lacked labile and recalcitrant fraction data from original soil. The carbon and $\delta^{13}C$ values of 1999 soil at 30–60 cm (combining the 30–45 and 45–60 cm soil samples) were used to calculate MRT.

2.6. Statistical analysis

The general liner model for two-factor experiment was employed to test the differences of the resulting parameters affected by the first factor of CO_2 and the second factor of water in this study. The parameters such as total SOC (C_t), recalcitrant carbon (C_r), labile carbon (C_l), and the ratios of C_r/C_t and C_l/C_t were tested within a depth interval and on the values averaged across the whole soil profile (0–100 cm) as well. The changes in new and old C_r and C_l contents were tested only on the average of the 0–100 cm depth. MRT was tested within the 0–15, 15–30, and 30–60 cm depth intervals between the treatments. To assess the relationships between MRT of bulk SOC and ratios of C_r and C_l to C_t, linear regression models for each calculation of MRT were fitted through all data points derived from FACE and Control, wet and dry, and all depths. All error estimates presented in tables and error bars in figures are standard deviation (Stdev). Statistically significant differences were considered at the $\alpha \leqslant 0.05$ probability levels. All statistical procedures were conducted using SPSS statistical software (Version 13.0, SPSS Inc., Chicago, IL).

3. Results

3.1. Labile and recalcitrant pools

Total SOC, ranging from the mean of 5.1 g kg^{-1} in the top layer to 1.6 g kg^{-1} in the deep layer, was not significantly different at the comparable layers between ambient and elevated CO_2 treatments under either water regime after the two-year FACE experiment, but the labile and recalcitrant C pools were significantly shift by elevated CO_2 (Table 1, where C_t is the sum of the C in the labile and recalcitrant pools). The values of 1999 SOC in Table 3 measured with bulk soil were higher than C_t in the Table 1 by an average of 1.6 g kg^{-1}. This difference was caused by the different procedures and was assumed not to introduce an appreciable bias on the results of samples with CO_2 and water treatments (Paul et al., 2006). Viewed within the C in the soil sub-pools, elevated CO_2 significantly decreased the C_l content of the whole soil profile (0–100 cm) on average from 1.53 g kg^{-1} in CD to 1.29 g kg^{-1} in FD by 15.7%, and from 1.60 g kg^{-1} in CW to 1.31 g kg^{-1} in FW by 18.1%. In contrast, elevated CO_2 increased C_r content on average from 1.33 g kg^{-1} in CD to 1.60 g kg^{-1} in FD by 20.3% under water-deficient condition, and slightly increased C_r under water-adequate condition. The shift in C_r and C_l resulted in significantly higher C_r/C_t ratios (0.55 and 0.56 in FD and FW, respectively) and lower C_l/C_t ratios (0.45 and 0.49 in FD and FW, respectively) under elevated CO_2 than their comparable Control treatments (the C_r/C_t ratios of 0.47 and 0.51, and the C_l/C_t ratios of 0.54 and 0.49 in CD and CW, respectively).

Down the soil profile, the C_r/C_t ratios overall increased from 0.41 to 0.64 with depth at 0–60 cm, whereas the C_l/C_t decreased from 0.59 to 0.36 with depth, but no significant difference was found at the equivalent layers between the treatments. Interestingly, from 60 to 100 cm, the C_l/C_t ratio unexpectedly increased whereas C_r/C_t ratio decreased. Generally, water alone was not found to significantly affect total carbon content and the change in labile and recalcitrant pools at either CO_2 level. Except for the difference in C_r between CD and CW treatments, statistical

Table 1

Distribution of mean ± 1 standard deviation (g kg^{-1}) of total SOC (C_t), labile C (C_l), recalcitrant C (C_r), and ratios of C_r/C_t and C_l/C_t in the soil profile (0–100 cm) in CD (Control: 360 μmol mol^{-1} + dry: deficient water supply), FD (FACE: 560 μmol mol^{-1} + dry), CW (Control + wet: ample water supply) and FW (FACE + wet) plots ($n = 3$) after 2-year C_4-sorghum free-air CO_2 enrichment (FACE) experiment

Treatment	Depth (cm)	C_t (g kg^{-1})	C_r (g kg^{-1})	C_l (g kg^{-1})	C_r/C_t ratio	C_l/C_t ratio
CD	0–15	5.06 ± 0.34	2.15 ± 0.93	2.91 ± 0.16	0.41 ± 0.13	0.59 ± 0.13
	15–30	4.52 ± 0.50	2.08 ± 0.66	2.44 ± 0.19	0.45 ± 0.11	0.55 ± 0.11
	45–60	2.48 ± 0.72	1.33 ± 0.71	1.15 ± 0.01	0.52 ± 0.14	0.48 ± 0.14
	80–100	1.81 ± 0.30	0.49 ± 0.15	1.32 ± 0.45	0.28 ± 0.13	0.72 ± 0.13
	Average[a]	2.86	1.33	1.53	0.47	0.54
FD	0–15	4.17 ± 0.83	2.22 ± 0.06	1.94 ± 0.97	0.55 ± 0.14	0.45 ± 0.14
	15–30	4.11 ± 0.01	2.17 ± 0.07	1.94 ± 0.15	0.51 ± 0.09	0.49 ± 0.09
	45–60	3.15 ± 0.02	2.00 ± 0.12	1.15 ± 0.14	0.64 ± 0.04	0.36 ± 0.04
	80–100	1.30 ± 0.01	0.54 ± 0.17	0.75 ± 0.16	0.42 ± 0.13	0.58 ± 0.13
	Average[a]	2.88	1.60	1.29	0.55	0.45
CW	0–15	5.49 ± 0.42	2.62 ± 0.39	2.88 ± 0.13	0.48 ± 0.04	0.53 ± 0.04
	15–30	4.33 ± 0.24	1.95 ± 0.21	2.38 ± 0.18	0.45 ± 0.04	0.55 ± 0.04
	45–60	3.04 ± 0.04	1.71 ± 0.09	1.33 ± 0.05	0.56 ± 0.02	0.44 ± 0.02
	80–100	1.81 ± 0.76	0.98 ± 0.33	0.83 ± 0.03	0.50 ± 0.19	0.50 ± 0.19
	Average[a]	3.23	1.63	1.60	0.51	0.49
FW	0–15	5.61 ± 0.81	3.18 ± 0.42	2.43 ± 0.04	0.57 ± 0.02	0.43 ± 0.02
	15–30	3.82 ± 0.24	2.08 ± 0.44	1.74 ± 0.20	0.58 ± 0.15	0.42 ± 0.15
	45–60	2.63 ± 0.31	1.71 ± 0.22	1.07 ± 0.15	0.59 ± 0.11	0.41 ± 0.11
	80–100	1.54 ± 0.38	0.67 ± 0.28	0.87 ± 0.11	0.43 ± 0.07	0.57 ± 0.07
	Average[a]	2.96	1.65	1.31	0.56	0.44
p-levels	CO_2	ns	ns	**	**	**
	Water	ns	+	ns	ns	ns
	CO_2*Water	ns	ns	ns	ns	ns
	CO_2*Depth	ns	ns	ns	ns	ns
	CO_2*water*Depth	ns	ns	ns	ns	ns

[a] Average—the mean SOC content (g kg^{-1}) of 6 depths adjusted with the soil bulk density and thickness of each layer was calculated as C(g kg^{-1}) = $\sum_{i=1}^{n}(L_i*\rho_{bi}*C_i)/\sum_{i=1}^{n}(L_i*\rho_{bi})$, where, L_i is thickness (cm) of ith layer; ρ_{bi} is soil bulk density (g cm^{-3}) in ith layer; C_i is soil organic carbon content (C: g kg^{-1}) at ith layer; $n = 6$. The data for the 30–45 and 60–80 cm layers used in this calculation were interpolated, using the average of the values of two adjacent layers. The significant differences affected by the factors of CO_2, water and depth, and by their interactions are indicated as "+"—$p < 0.1$; "**"—$p < 0.01$, and "ns"—not significant.

analysis revealed no significant difference in C_l and the ratios of C_r/C_t and C_l/C_t between CD and CW, and no significant differences in C_r, C_l, C_r/C_t and C_l/C_t between FD and FW. Also, there were no significant interactive effects of CO_2 with water or/and depth on the shifting of the labile and recalcitrant carbon pools (Table 1).

3.2. $\delta^{13}C$ values

The $\delta^{13}C$ values of labile and recalcitrant C in the soil profile were affected by the input of new C_4-sorghum residues. As a result, both labile and recalcitrant carbons were more enriched in ^{13}C than the initial SOC (Fig. 1). The $\delta^{13}C$ values of both labile and recalcitrant C largely increased by the averages of 1.74‰ and 0.45‰, respectively, compared to the $\delta^{13}C$ values of 1998 bulk SOC. Over the soil profile, surface soil $\delta^{13}C$ averaged across dry and wet showed the maximum differences between initial SOC and labile and recalcitrant carbon of 3.26‰, 0.85‰, respectively, and the differences declined with depth.

Within both pools, elevated CO_2 resulted in less negative $\delta^{13}C$ than that of ambient CO_2, but significant differences between FACE and Control recalcitrant C pools and labile C pools were only found in surface soil horizon (Fig. 1). For the labile C pool (Fig. 1A and B), $\delta^{13}C$ values at 0–30 cm in FACE plots increased from −22.68‰ of 1998 SOC to −19.13 and −19.05‰ in FACE dry and wet sites, respectively. Compared with the $\delta^{13}C$ values of −19.71 and −19.70‰ in Control dry and wet plots, the differences between FACE and Control were about 0.58‰ and 0.65‰ under dry and wet conditions, respectively. For the recalcitrant C pool (Fig. 1C and D), the $\delta^{13}C$ values were close to those of 1998 SOC, and the differences in $\delta^{13}C$ between FACE and Control was 0.78‰ and 0.42‰ in the surface soil under wet and dry conditions, respectively. At 30–60 cm, the differences in the $\delta^{13}C$ values of two soil sub-pools between FACE and Control were not significant under either wet or dry condition.

3.3. New and old carbon

Distributions of the new and old carbons in labile and recalcitrant pools were influenced by elevated CO_2 and water regimes, but no CO_2 × water interaction effects were found (Table 2, Fig. 2 and 3). Elevated CO_2 significantly reduced both new and old C_l by an average of 13.7% and 13.4%, respectively, relative to ambient CO_2, and significantly increased both new C_r by 36.4% and 25.0%,

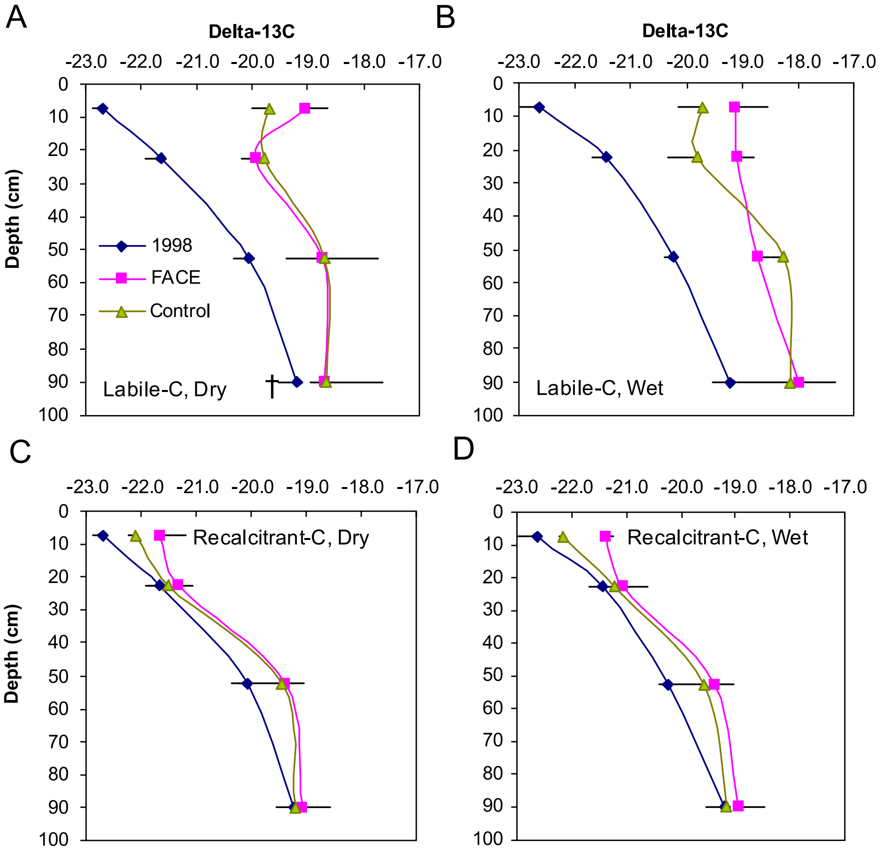

Fig. 1. Mean $\delta^{13}C$ values for labile carbon (A and B), and recalcitrant carbon (C and D) under dry (A and C) and wet (B and D) in soils (0–100 cm) sampled from FACE (square) and Control (triangle) plots at the end of 1999 growing season compared with the mean $\delta^{13}C$ values of 1998 (diamond) bulk SOC of the soils collected at the beginning of FACE experiment. Error bars indicate one standard deviation, $n = 3$ (Because we did not collect soil down to 100 cm in 1998, the $\delta^{13}C$ values of 1998 soil at the depth of 80–100 cm were substituted with the $\delta^{13}C$ values of 1999 soil collected at 80–100 cm from a fallow plot in which no plants have grown since 1998).

Table 2
Means ± 1 standard deviation of total SOC, new and old labile C, and new and old recalcitrant C in the CD, FD, CW, and FW soils at the depth of 0–100 cm

Treatment	Total SOC* ($\mathrm{kg\,m^{-2}}$)	Labile carbon (C_l) ($\mathrm{kg\,m^{-2}}$)		Recalcitrant carbon (C_r) ($\mathrm{kg\,m^{-2}}$)	
		New C_l	Old C_l	New C_r	Old C_r
CD	3.83 ± 0.46	0.34 ± 0.04	1.71 ± 0.06	0.08 ± 0.02	1.71 ± 0.38
FD	4.02 ± 0.41	0.28 ± 0.03	1.52 ± 0.20	0.10 ± 0.02	2.12 ± 0.25
FD/CD	1.05	0.81	0.89	1.35	1.24
CW	4.46 ± 0.39	0.41 ± 0.05	1.79 ± 0.02	0.11 ± 0.01	2.14 ± 0.34
FW	4.26 ± 0.44	0.37 ± 0.08	1.51 ± 0.22	0.15 ± 0.01	2.23 ± 0.17
FW/CW	0.96	0.90	0.84	1.39	1.04
p-levels					
CO_2	ns	*	***	***	ns
Water	ns	*	ns	**	ns
CO_2*water	ns	ns	ns	ns	ns

The data were the average of three replicates in units of kg per square meter. For each sample site, the data were calculated as: $C(\mathrm{kg\,m^{-2}}) = \sum_{i=1}^{n}(L_i * \rho_{bi} * C_i) * 10$, where, L_i is thickness (cm) of ith layer; ρ_{bi} is soil bulk density ($\mathrm{g\,cm^{-3}}$) at the ith layer; C_i is organic C content (C%) of the ith layer; $n = 6$. Here, because we did not analyze the soils collected from 30–45 cm and 60–80 cm, the data used in the above equation for these two layers were interpolated, using the average of the values of adjacent two layers.

The significant differences affected by the factors of CO_2, water and their interactions are indicated as "*"—$p < 0.05$; "**"—$p < 0.01$; "***"—$p < 0.001$; and "ns"—not significant.

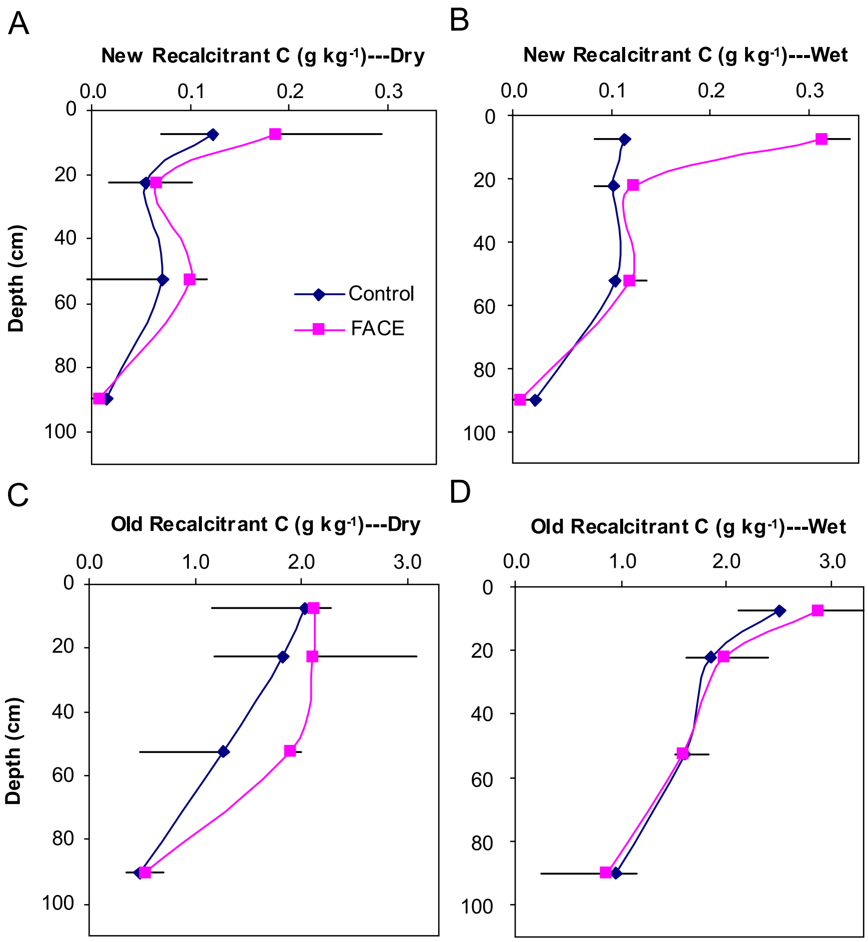

Fig. 2. Distribution of the means of recalcitrant carbon derived from new sorghum residues (A: dry and B: wet) and old pre-experiment SOC (C: dry and D: wet) in the soil profiles (0–100 cm) of FACE (square) and Control (diamond) plots. Error bars represent one standard deviation, $n = 3$.

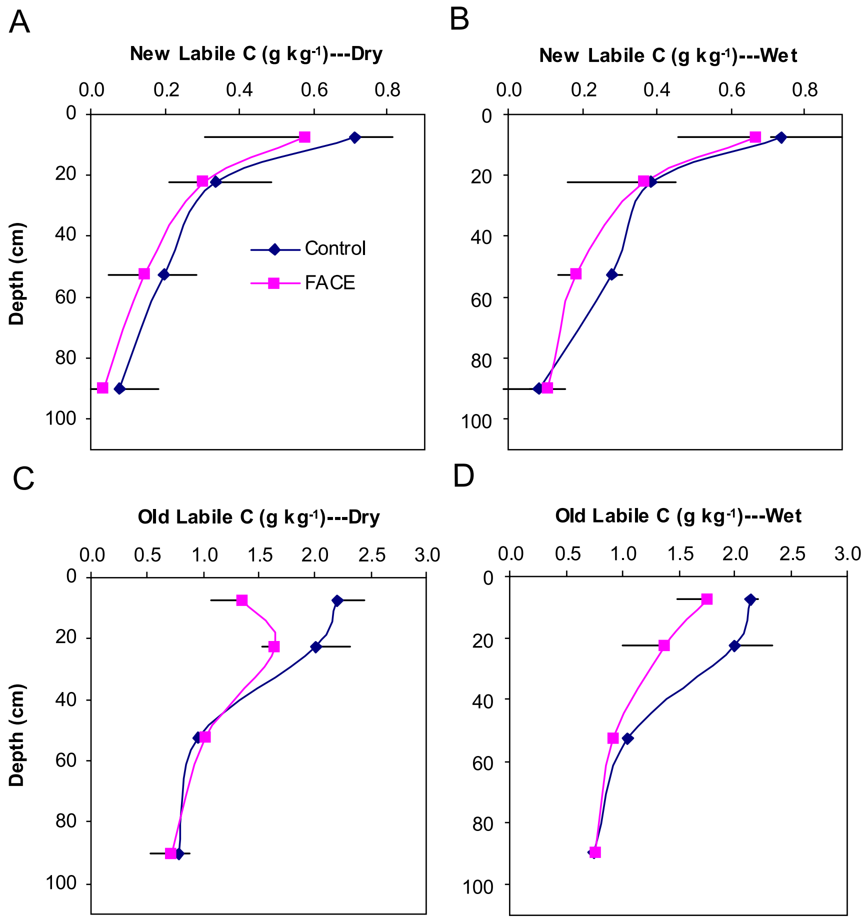

Fig. 3. Distribution of the means of labile carbon derived from new sorghum residues (A: dry and B: wet) and old preexperiment SOC (C: dry and D: wet) at the soil profile (0–100 cm) in FACE (square) and Control (diamond) plots. Error bars represent one standard deviation, $n = 3$.

respectively, under water-adequate and water-deficient conditions. Viewed down soil profile, the significant CO_2 effects occurred only at surface soils, not in the deeper soil layers (Fig. 2 and 3). Water-adequate treatment significantly increased new C_l and new C_r, but no effects on old C_l and C_r compared to water-deficient treatment. The interaction of CO_2 with water had no statistic effect on all parameters (Table 2).

3.4. *Mean residence time (MRT)*

Overall, MRT of bulk SOC was much shorter in the surface soils than the subsurface soils, and a significant CO_2 effect on MRT occurred only at the surface soils, which was interacted with water conditions (Table 3). At 0–15 cm, the rate constant (k) for loss of bulk SOC was much lower in high CO_2 plots than ambient CO_2 plots, and consequently MRT was higher in elevated CO_2 plots (10 yr under water-deficient conditions and 11 yr under water-adequate conditions) than ambient CO_2 plots (9 yr under water-deficient and 7 yr under water-adequate). The difference under water-deficient conditions was not significant. At 15–30 cm, MRT was about 35 yr in the elevated CO_2 soils, significantly higher than 14 yr in the ambient CO_2 soils under both water-adequate and water-deficient conditions. At 30–60 cm, the decay rate of SOC was much lower. As a result, the MRT was much longer at the deeper soils, but there are no significant differences between elevated and ambient CO_2 treatments. Water alone did not affect the MRT of bulk SOC.

4. Discussion

4.1. *Shift of labile and recalcitrant pools*

Although we did not find a significant difference of total SOC between elevated and ambient CO_2 treatments after the two-year FACE experiment, we did detect the significant shifts of SOC between labile and recalcitrant C pools. Overall, carbon in the labile and recalcitrant pools each represented about 50% of total SOC. However, the elevated CO_2 treatment resulted in more C in the recalcitrant pool and less C in the labile pool. More specifically, the interaction of elevated CO_2 plus deficient-water supply showed a greater difference in the shift of labile and recalcitrant C pool compared to elevated CO_2 plus ample-water supply (Table 1). Under water-deficient condition, elevated CO_2 increased C_r by 15.6%, and

Table 3
Averaged SOC decay rate (k: yr^{-1}) and mean residence time (MRT: yr) of bulk soil organic matter at soil profile (0–60 cm), as determined by $\delta^{13}C$ values and SOC content (mean ± 1 standard deviation, $g\,kg^{-1}$) in 1998 and 1999 FACE experiments

Treatment	Depth (cm)	SOC ($g\,kg^{-1}$)		Fraction of 1998 C in 1999 SOC	k	MRT ($1/k$)
		1998	1999	(f)	(yr^{-1})	(yr)
CD	0–15	7.06 ± 1.59	6.54 ± 0.22	0.937	0.1135	9
	15–30	5.76 ± 1.02	5.90 ± 1.16	0.888	0.0737	14
	30–60	4.01 ± 0.73	4.10 ± 0.48	0.967	0.0086	116
FD	0–15	7.03 ± 0.71	6.93 ± 0.99	0.890	0.1044	10
	15–30	5.35 ± 0.89	5.60 ± 0.24	0.923	0.0279	36
	30–60	4.12 ± 0.89	4.21 ± 0.84	0.966	0.0093	107
CW	0–15	7.28 ± 0.80	6.71 ± 0.55	0.916	0.1353	7
	15–30	5.87 ± 0.77	5.69 ± 0.38	0.941	0.0733	14
	30–60	4.28 ± 0.26	4.28 ± 0.54	0.987	0.0105	96
FW	0–15	6.91 ± 0.62	7.18 ± 0.35	0.856	0.0942	11
	15–30	5.40 ± 0.95	5.85 ± 0.70	0.890	0.0282	35
	30–60	4.20 ± 0.49	4.30 ± 0.34	0.965	0.0093	108

Main effect analysis on MRT	p-levels		
	CO_2	Water	$CO_2{*}$water
0–15	***	ns	***
15–30	***	ns	ns
30–60	ns	ns	ns

The significant differences of MRT within a depth interval affected by CO_2, water and their interactions are indicated as "***"—$p < 0.001$, and "ns"—not significant.

decreased C_l by 27.3%, compared to the ambient CO_2, whereas, under water-adequate conditions, elevated CO_2 slightly increased C_r by 5.9% and decreased C_l by 21.6%. This indicates that water stress enhanced the CO_2 effect on the shift of carbon between the labile and recalcitrant pools. These trends were consistent with the conclusions from above-ground study in this FACE experiment by Ottman et al.(2001), who pointed out that the great benefit from long-term CO_2 enrichment occurs when water supply is non-limiting, but the enhancement of relative change increases substantially when water is deficient.

Viewed over the soil profile from surface to 60 cm, C_r/C_t generally increased from 0.41 to 0.64, whereas the C_l/C_t decreased from 0.59 to 0.36 with the depth. This pattern agrees with Paul et al. (2001a) who found in soils collected from South Charleston, OH, that the proportion of non-hydrolyzable C increased with increasing depth, from 49% in the surface soil to 63% in subsurface horizons. Interestingly, at the depth of 80–100 cm, the C_l/C_t ratio unexpectedly increased whereas C_r/C_t ratio decreased, indicating a large proportion of hydrolyzable C was precipitated in the deeper soil. This phenomenon perplexes us and its cause may relate to organic matter contributions from deeper roots or the leaching of soluble organic C down to this depth. Considering the long turnover time at the depth, the large portion of labile C is probably and mainly parent material-derived SOC. C_r and C_l results for each layer under ambient CO_2 were not significantly different from those found under elevated CO_2. This may be because of the short-term nature of the experiment. However, we found the largest differences in C_r/C_t and C_l/C_t ratios between FACE and Control at the surface soils. For example, FW was significantly different in C_r/C_t from CW at 0–30 cm, not below 30 cm, suggesting elevated CO_2 had a stronger effect on the dynamics of SOC at surface layer than in subsurface layers.

4.2. *Implication of the $\delta^{13}C$ signature*

After the 2-year FACE experiment, the $\delta^{13}C$ values of both labile and recalcitrant C were more enriched in ^{13}C compared to the isotopic carbon composition of initial SOC. This suggests that ^{13}C-rich sorghum residues were incorporated into the both pools. Sorghum as a C_4-plant is ^{13}C-enriched with $\delta^{13}C$ values of −10.35, −11.80, −10.92, and −11.89‰ for the whole sorghum plants derived from FW, CW, FD, and CD, respectively (detail sorghum sample collection and isotopic measurement were described in Leavitt et al., in revision). As mentioned, the FACE field prior to the experiment was frequently cultivated with C_3 crops that are ^{13}C-depleted, resulting in much more negative $\delta^{13}C$ values of SOC, −22.68‰ to −19.21‰ from surface to subsurface soil. Less negative $\delta^{13}C$ value of SOC indicate greater contribution from sorghum residues. A larger $\delta^{13}C$ difference was found between labile C and 1998 original C than between recalcitrant C and 1998 C, indicating that new sorghum residues were the major contributor to the labile C pool, whereas original SOC was a major component of recalcitrant C pool.

Nevertheless, the difference in the $\delta^{13}C$ values of the labile pool between FACE and Control was smaller than the difference in $\delta^{13}C$ values between FACE and Control sorghum residues, indicating that a non-negligible portion of native old SOC transformed into the labile C pool and diluted the $\delta^{13}C$ values. Particularly at 30–60 cm, the difference in $\delta^{13}C$ values of the labile C pool between FACE and Control were not significant, implying less new sorghum residue and more original SOC was contributed to this pool. For the recalcitrant C pool, the $\delta^{13}C$ values were very close to those of original SOC, meaning a major component of recalcitrant C pool was original old SOC. Although the difference in $\delta^{13}C$ between FACE and Control recalcitrant pools was not significant, C in the FACE recalcitrant pool was more enriched in ^{13}C than that in the Control recalcitrant pool throughout the whole soil profile, implying elevated CO_2 affected the recalcitrant C pool, probably through effects on sorghum root growth and a change in residue chemistry that might have a permanent effect on total SOC storage.

4.3. *New and old carbon translocation*

Above-ground study in this FACE experiment did not find a significant difference in the quantity of sorghum residue input to ground between elevated and ambient CO_2 treatments (Ottman et al., 2001; Wall et al., 2001). However, the translocation of new and old carbon among labile and recalcitrant pools was significantly influenced by elevated CO_2 (Table 2 and Figs. 2 and 3). Viewed across all treatments, we found that elevated CO_2 resulted in both more new C and old C in the recalcitrant pool, and less new C and old C in the labile pool compared to the ambient CO_2 treatments, suggesting the carbon derived from sorghum residues transfers into the recalcitrant pool from the labile C pool (Table 2, and Fig. 2). This new C_r carbon could be dominated by sorghum-residue-derived resistant compounds such as lignin and non-lignin phenolics (Rovira and Vallejo, 2002). This agrees with the findings from sorghum chemistry assays, which showed that the resistant compounds of sorghum tissues such as lignin, and non-lignin phenolics increased with elevated CO_2 (by 7.0–3.4%, unpublished in Cheng's (2005) dissertation). However, the increment of new C in the recalcitrant C pool is much higher than the chemical change of those resistant compounds in sorghum tissues. This may be explained if the initial lignin and non-lignin phenolic concentrations in sorghum tissues not only directly added resistant material to new soil C_r but also formed other recalcitrant compounds, such as humic substances, by synthetic interaction with labile compounds (Campbell et al., 1967). This hypothesis is supported by sharply decreasing new carbon in the labile pool (17.6% and 9.8% reduction in FD and FW labile pools, respectively). Major components of new labile carbon should be easily degradable compounds originating from sorghum residues such as amino acids and soluble carbohydrates, which also did not decline in sorghum tissues as much as in soil labile C pool under elevated CO_2. This further shows that under elevated CO_2, labile carbon was probably transferred to the recalcitrant pool in the soil or degraded into CO_2 released out of the soil.

Similar to new C, old C in the recalcitrant pool was higher under elevated CO_2 than under ambient CO_2 conditions (Fig. 3 and Table 2). On average, old C in the recalcitrant pool was 24.0% significantly higher under elevated CO_2 and deficient water and slightly higher with ample water than their corresponding Controls. In contrast, old C in the labile pool was on average 11.1% and 15.6% significantly lower in FD and FW treatments, respectively, than their comparable Controls. This indicates that mineralization of native SOC was retarded under CO_2 enrichment, probably because of the depression of soil microbial activity with more resistant substrate input under elevated CO_2, or alternatively, because easily decomposable substrates stimulated microorganism activity to accelerate the decomposition of old SOC under ambient CO_2. Furthermore, the results imply that elevated CO_2 effects on dynamics of SOC depend more on plant residue quality than on quantity. Because most of the new sorghum residue was input at 0–30 cm, elevated CO_2 effects on the shift of labile and recalcitrant C pool most significantly occurred in the surface soils.

4.4. *Longer MRT in the FACE soil*

The longer MRT in the surface soils of the FACE plot than Control plot was probably related to two processes. First, during the growing season, elevated CO_2 increased the availability of contemporaneous rhizodeposits, such as root exudates, sloughing root caps and dead fine roots, thereby redirecting microbial decomposition away from old SOC. This mechanism is consistent with the hypotheses of Goudriaan and de Ruiter (1983) who proposed that increased inputs of soluble, easily decomposed C as a consequence of higher atmospheric CO_2 could satisfy the substrate preferences of soil microbes for easily decomposable substrates, which would consequently retard the decomposition of native soil organic matter. The increase in root growth and root exudation stimulated by elevated CO_2 and decrease in turnover time of native SOC has been broadly reported by many researchers (Goudriaan and de Ruiter, 1983; Chaudhuri et al., 1986; Van Veen et al., 1991; Rogers and Prior, 1992). Second, after the growing season, above-ground biomass of sorghum, except for grain, was returned to the soil. Those new soil organic materials contained more secondary and carbon-based structural compounds when sorghum was grown under elevated CO_2. These lower quality residues could not provide a lot of energy for microorganisms and consequently reduced microbial activity in FACE

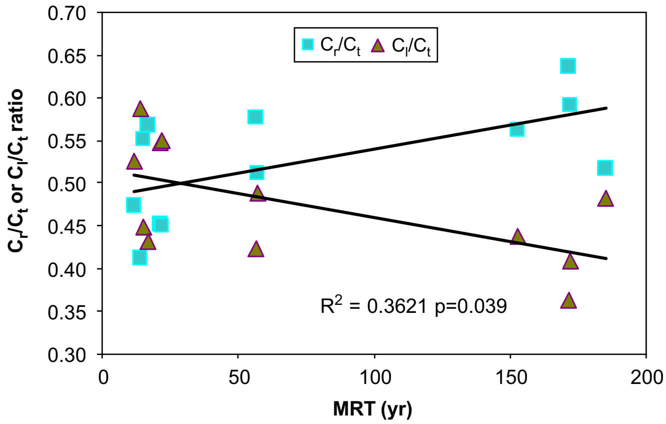

Fig. 4. Regression of bulk SOC MRT with the ratios of C_r/C_t (squares) and C_l/C_t (triangles).

soils (although this seems in conflict with the findings of Rillig et al. (2001) who reported the soil in this FACE experiment contained more fungi than Control soil). As a result, the breakdown of old organic C decreased and MRT of FACE SOC increased. This mechanism is corroborated by the studies of other researchers who have demonstrated that elevated CO_2 reduces substrate quality and increases MRT of SOC (e.g., Berendse et al., 1987; McLaugherty and Berg, 1987; Nicolai, 1988; Lewis and Yamamoto, 1990).

4.5. Correlation MRT with C pools

The MRTs were calculated for the bulk soils collected at the beginning of 1998 and at the end of 1999 growing seasons. Previous studies by Leavitt et al. (1996) and Paul et al. (2001a) have shown that carbon in labile pool is much younger than bulk SOC, whereas carbon in recalcitrant pool is much older than bulk SOC. To confirm their findings with our results, the MRT of bulk soil was correlated with ratios of C_r/C_t and C_l/C_t (Fig. 4). The results show a significant positive correlation ($r^2 = 0.36$, $P < 0.05$) between MRTs and C_r/C_t ratios, and a significant negative correlation ($r^2 = 0.36$, $P < 0.05$) between MRTs and C_l/C_t ratios, confirming carbon in the recalcitrant pool is more stable than in the labile pool. Also, this correlation revealed that C_r/C_t and C_l/C_t may be good indicators to evaluate the stability of total SOC. It should be pointed out that MRT of total SOC calculated by $\delta^{13}C$ might be much younger than the real age measured by ^{14}C. Paul et al. (2001a), after the analysis of the relationship between $\delta^{13}C$ MRT and ^{14}C age, reported that total SOC has an MRT calculated from ^{14}C that is approximately 176 times the square root of MRT derived from $\delta^{13}C$. In the present study, elevated CO_2 significantly increased the C_r/C_t ratio and the MRT of total SOC calculated by $\delta^{13}C$, suggesting that carbon fixed by the plant and entering the soil via plant residues under elevated CO_2 environment may reside there for hundreds of years more than the carbon input under current background CO_2 levels.

5. Conclusion

Stable-carbon isotopic tracing ($\delta^{13}C$) provided a powerful approach for probing dynamics of labile and recalcitrant C pools in soil profiles of the Arizona Maricopa sorghum FACE experiment. Higher recalcitrant C content and lower labile C content in the soils were detected under elevated CO_2 relative to ambient CO_2 treatments, suggesting that SOC under elevated CO_2 becomes more stable against chemical and biological degradation. Separation of old and new C from each pool with an isotopic mass balance model revealed that both new C and old C in the recalcitrant C pool under elevated CO_2 increased compared to ambient Control, confirming that change in quality of sorghum residues produced under elevated CO_2 plays a vital role in regulating the dynamics of soil C pools (Heal et al., 1997; Cotrufo et al., 1998), and also indicating that highly stable residues of FACE sorghum were a major contributor to the recalcitrant pool, not only because of the direct addition of new resistant organic C to recalcitrant pool but also because of indirect reduction of old SOC decomposition. The significant increase in MRT of total SOC and its strong positive correlation with C_r/C_t provide further evidence that elevated CO_2 may slow down breakup of old SOC and build up new recalcitrant C in the soil. Impact of water alone on bulk SOC or on labile and recalcitrant pools was not significant. However, water stress interacting with CO_2 enhanced the shift of the SOC from the labile pool to recalcitrant pool. All the significant differences in the soil C pools influenced by elevated CO_2 or by the interaction of CO_2 with water occurred at the surface soils (0–30 cm). Overall, in our study, quantification of labile and recalcitrant carbon pools suggested that altered soil carbon transformation and MRT under elevated CO_2 may ultimately result in profound change in long-term net carbon movement from the atmosphere to agroecosystem.

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