Elevated Atmospheric CO$_2$ Impacts Carbon Dynamics in a C$_4$-Sorghum-Soil Agroecosystem

— An Application of Stable Carbon Isotopes ($\delta^{13}$C) in Tracing the Fate of Carbon in the Atmosphere-Plant-Soil Ecosystem

by

Li Cheng

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As members of the Dissertation Committee, we certify that we have read the dissertation prepared by Li Cheng entitled “Elevated Atmospheric CO$_2$ Impacts on Carbon Dynamics in a C$_4$-Sorghum-Soil Agroecosystem — An Application of Stable Carbon Isotopes ($\delta^{13}$C) in Tracing the Fate of Carbon in the Atmosphere-Plant-Soil Ecosystem” and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy (Ph. D.).

Date: April 15, 2005
Steven W. Leavitt

Date: April 15, 2005
James Walworth

Date: April 15, 2005
Dean Martens

Date: April 15, 2005
Allan Matthias

Date: April 15, 2005
Hinrich Bohn

Final approval and acceptance of this dissertation is contingent upon the candidate’s submission of the final copies of the dissertation to the Graduate College.

I hereby certify that I have read this dissertation prepared under my direction and recommend that it be accepted as fulfilling the dissertation requirement.

Date: April 15, 2005
Dissertation Director: Steven W. Leavitt, James Walworth
STATEMENT BY AUTHOR

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SIGNED: Li Cheng
DEDICATION

This dissertation is dedicated to my wife, daughter, parents and all relatives for their love, care and encouragement.
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ABSTRACT

Although a strong inter-dependence exists between atmospheric carbon dioxide (CO$_2$) and the terrestrial carbon (C) cycle, the response of plant-soil ecosystems to the rapid increase in atmospheric CO$_2$ is not well understood. My dissertation research focused on the impacts of elevated CO$_2$ on the carbon dynamics of plant-soil ecosystems, which were a major part of the overall C$_4$-sorghum Free-Air CO$_2$ Enrichment (FACE) experiment conducted by the University of Arizona and USDA at the Maricopa Agriculture Center, Arizona, USA, in 1998 and 1999. In the experiment, sorghum (*Sorghum bicolor* (L) Mõench) crop was exposed to elevated CO$_2$ (“FACE”: ca. 560 µmol mol$^{-1}$) and ambient CO$_2$ (“Control”: ca. 360 µmol mol$^{-1}$) interacting with well-watered and water-stressed treatments.

The results from my study showed that the seasonal mean soil respiration rate measured in elevated CO$_2$ plots over two growing seasons was 3.3 µmol m$^{-2}$ s$^{-1}$, i.e., 12.7% higher than the 2.9 µmol m$^{-2}$ s$^{-1}$ in ambient CO$_2$ plots. The increased respiration mainly resulted from the stimulated root respiration under elevated CO$_2$, which increased 36.1% compared to that under ambient CO$_2$. Measured changes in sorghum residue biochemistry caused by CO$_2$ were detected, with decrease of amino acids and hemicellulose carbohydrates by 7% and 8%, respectively, and increase of cellulose carbohydrates and lignin by 49% and 5%, respectively. Phenolics were only significantly higher in FACE roots. The C:N ratio of sorghum tissues was not affected by elevated CO$_2$, but was substantially lower under water stress.

The laboratory incubation showed that an average of 7.3% significantly less respired CO$_2$ was released from the FACE-tissue-amended soil than the Control-
tissues-amended soil over the full 79-d incubation period. Non-lignin phenolics ($r^2 = 0.93, p = 0.002$), and lignin ($r^2 = 0.89, p = 0.004$) were found to be the most important factors related to the sorghum tissue decomposition. Highly stable residues of FACE sorghum input to the soil resulted in the increase of the recalcitrant C pool and the decrease of the labile C pool. As a result, mean residence time of SOC in FACE field plot increased compared to that in Control plot, suggesting that the SOC under elevated CO$_2$ was more stable against decomposition.
1. CHAPTER I: INTRODUCTION

1.1. The Problem and Its Origin

1.1.1. The Problem

Atmospheric concentration of carbon dioxide (CO$_2$) is predicted to double in the next century; but the response of terrestrial ecosystems to rising CO$_2$ is still not fully understood (IPCC, 2001). The problem, carefully addressed in this dissertation, is to determine the impacts of elevated CO$_2$ on the carbon cycle in plant-soil ecosystems. The resolution of the problem requires the detailed examination of a number of specific plant/soil parameters in response to elevated atmospheric CO$_2$, which include:

(1) Soil respiration, namely soil microbial activity, which determines soil organic carbon (SOC) decomposition. This effort requires accurate separation of root respiration from the mineralization of old SOC by microbial activity.

(2) Plant residue biochemistry, which is one of several important factors regulating soil microbial growth and activity.

(3) The relationship between residue decomposition and residue chemical composition. This effort requires an understanding of which residue chemical compounds are most related to residue decomposition rate.

(4) The structure and size of soil carbon sub-pools, which provide evidence to evaluate the long-term effect of CO$_2$ on carbon sequestration in the terrestrial ecosystems. In this effort, the soil carbon pool must be properly partitioned into different sub-pools on the basis of stability.
My research has investigated and characterized each of these parameters to clarify the responses of soil microbial activity, plant chemistry and the soil carbon pool --- the major components of terrestrial ecosystems --- to elevated atmospheric CO$_2$.

In this study, I hypothesized the following:

*Hypothesis 1.* Elevated CO$_2$ will stimulate soil microbial growth and activity as a consequence of the CO$_2$ effects on plant physiological processes, thereby increasing soil respiration.

*Hypothesis 2.* Increased plant root respiration will be accompanied by an increased SOM mineralization rate because of high microbial activity.

*Hypothesis 3.* Stimulation of old SOC decomposition because of addition of new fresh plant residue will be related to not only the quantity of the new organic matter input but also to the chemical composition of input organic matter.

*Hypothesis 4.* Elevated CO$_2$ provides excess carbon above plant growth requirements that will be accumulated in total nonstructural carbohydrates and carbon-based secondary and structural compounds.

*Hypothesis 5.* Substrate quality is one of the most important factors regulating soil decomposition processes, and the decomposition rate of FACE (free-air CO$_2$ enrichment) sorghum tissues will be reduced if more recalcitrant carbon-based structural and secondary compounds are produced under elevated CO$_2$.

*Hypothesis 6.* Elevated CO$_2$ should increase the size of the long-term soil carbon pool because more resistant plant residues are input to the soil under elevated CO$_2$. This could play a vital role in the potential for long-term C sequestration in terrestrial agroecosystems in response to rapidly increased atmospheric CO$_2$. 
1.1.2. Origin

Of all the changes of the global environment that are occurring, the rapid increase in the CO2 concentration of the atmosphere is considered to be the most important long-term change and the most troublesome because it has both direct and indirect impacts on the operation of the Earth’s climate. The atmospheric CO2 concentration has drastically risen over 31% from a pre-industrial concentration of ca. 280 μmol mol\(^{-1}\) to 370 μmol mol\(^{-1}\) in the present atmosphere, and is currently increasing at the rate of 1.5 μmol mol\(^{-1}\) year\(^{-1}\) (IPCC, 2001; Keeling and Whorf, 2002), which is 10-100 times faster than the largest rate of natural CO2 change in the last 420,000 years (Falkowski et al., 2000). This peculiar increase is mainly attributed to human activities, e.g. the combustion of fossil fuels and deforestation. It is predicted that atmospheric CO2 concentration will reach 700 μmol mol\(^{-1}\) by the end of the 21\(^{st}\) Century (Houghton et al., 1996). Carbon dioxide is an important greenhouse gas (GHG), with a current radiative forcing of 1.46 W m\(^{-2}\), much higher than the forcing by other anthropogenic GHGs in the atmosphere (e.g. the radiative forcing of CO2 is 2.9 times larger than that of CH4 and 9.7 times larger than that of N2O). The anthropogenic enrichment of GHGs in the atmosphere and the cumulative radiative forcing of all GHGs have led to an increase in the average global surface temperature of 0.6 °C since the late 19\(^{th}\) Century, with the current warming rate of 0.17 °C decade\(^{-1}\) (IPCC, 2001). Furthermore, the latest global circulation models predict a 1.5-2 °C increase in temperature when atmospheric CO2 concentration is doubled (Houghton et al., 1990). The rate of increase of the global mean temperature has exceeded the critical rate of 0.1 °C decade\(^{-1}\), above which the ecosystems cannot adjust (Lal, 2004). Higher temperature has dangerous consequences,
e.g. rising sea level, more frequent flooding, greater frequency of droughts and wildfires, increasing disease, species extinction and loss of ecosystems. One of the most direct consequences of elevated CO\textsubscript{2} may be to alter many ecosystem processes, of which the most important one is carbon cycling in terrestrial ecosystems.

Humans emit 7.9 Pg C (1Pg = petagram = billion ton) of carbon per year into the atmosphere. Of this 7.9 Pg C, 6.3±0.4 Pg C originate from fossil-fuel burning and cement production and 1.6±0.8 Pg C come from land-use change, and only about 3.2±0.1 Pg C year\textsuperscript{-1} accumulate in the atmosphere. Of the remainder, about 2.3±0.8 Pg C year\textsuperscript{-1} are absorbed by the oceans, and 2.3±1.3 Pg C is unaccounted for, but is believed to have been absorbed by terrestrial ecosystems (IPCC, 2001; Prentice, 2001).

It has been widely recognized that the terrestrial ecosystems play a critical role in regulating the concentration of CO\textsubscript{2} in the atmosphere. For example, seasonal variations in the amplitude of atmospheric CO\textsubscript{2} concentration, as shown in the Keeling Curve (Keeling et al., 1984) (also see Section 1.2.1.2), are significantly greater in the Northern Hemisphere than in the Southern Hemisphere, implicating the seasonal cycling of land plant photosynthetic activity and soil respiration as important factors controlling short-term atmospheric CO\textsubscript{2} levels. This indicates that the response of ecosystems to elevated atmospheric CO\textsubscript{2} constitutes a critical feedback within the global carbon cycle. Researchers have shown that elevated CO\textsubscript{2} often stimulates photosynthesis and results in increased primary products. The subsequent allocation and ultimate fate of this increased photosynthetically fixed carbon are important determinants of global carbon dynamics (Canadell et al., 1996). Currently, the mechanisms that govern the magnitude of net carbon uptake by the terrestrial biosphere
are unknown (Schimel et al., 2000). Although individual plants often respond to elevated CO$_2$ by fixing more carbon in photosynthesis and thereby increasing production of biomass, this does not necessarily result in long-term removal of CO$_2$ from the atmosphere. Much of the biomass is ultimately incorporated into soil as SOC (Fig. 1). Long-term removal requires the transfer of fixed carbon into long-term carbon pools in soil ecosystems such as the recalcitrant soil carbon pools (Körner, 2000).

![Carbon Cycle of Atmosphere-Plant-Soil Ecosystem](image)

Fig. 1. Carbon Cycle of Atmosphere-Plant-Soil Ecosystem. In the figure, NPP = “Net Primary Production”; LPM = “Labile Plant Materials”; RPM = “Recalcitrant Plant Materials”; SMB = “Soil Microbial Biomass”; HDM = “Partially Degraded Products”; POM = “Physically Protected Soil Organic Matter”; COM = “Chemically Protected Soil Organic Matter”. The figure shows that atmospheric CO$_2$ can be fixed by plant as biomass through CO$_2$ assimilation. Most of the biomass returns to the atmosphere as CO$_2$ by plant photo- and dark-respiration soon, which includes the root respiration from
belowground soil. What left in the plant is so called NPP. Most of the terrestrial NPP are ultimately incorporated into the soil. These organic materials can be divided into two types of materials based on their degradability---LPM and RPM. Once these materials input to the soil, they immediately go to the decomposition process by soil microorganisms. Most of them are gradated into CO$_2$ and returned to the atmosphere through soil respiration. For the rest of the plant materials that are not degraded to CO$_2$, they will go the SMB, HDM, POM, and COM pools, forming the different soil organic matters with various stabilities. The SMB and HDM pools can be together called as Labile Soil C Pool, because they easily go to the decomposition process in a short time, whereas the POM and COM pools can be together considered as Recalcitrant Soil C Pool, because they will stay in the soil for longer time before going to the decomposition process again. Whether or not the soil can sequester the atmospheric CO$_2$, namely net moving carbon from the atmosphere to the soil, depends on the increase in the size of the recalcitrant pool, which depends on not only the quantity but also the quality of the plant materials input to the soil.

Currently, whether the increasing atmospheric concentration of CO$_2$ increases net terrestrial ecosystem carbon storage is in intensive debate (Melillo et al., 1996; Norby et al., 2001). In recent years, much attention has been focused on anthropogenic increases in atmospheric CO$_2$ concentrations, because of its possible role in current global warming and in the photosynthetic uptake by vegetation. Researchers have examined a variety of plants and plant communities exposed to elevated CO$_2$ levels to improve our understanding of terrestrial ecosystem responses. Those studies have used open-top chambers, closed chambers, solar domes, growth cabinets, natural CO$_2$ springs, and free-air CO$_2$ enrichment (FACE) (Kimball et al., 2002). Many of these studies are also intrinsic isotopic tracer experiments. This is because stable carbon isotopic methods, available through mass spectrometry, provide a powerful approach to
trace carbon translocation in the system. In these experiments, the ratios of carbon-13 ($^{13}\text{C}$) to carbon-12 ($^{12}\text{C}$) and of carbon-14 ($^{14}\text{C}$) to $^{12}\text{C}$ in the added CO$_2$ are distinct from the ratios in atmospheric CO$_2$ (Leavitt et al., 1994). Plants grown in conditions of elevated CO$_2$ that contain such isotopic tracers will incorporate the tracer into photosynthetic products. Carbon inputs from isotopically labeled plant residues to the soils can be revealed by a shift in the isotopic composition of SOC. At present, the aboveground physiological response of plants to elevated atmospheric CO$_2$ has received considerable attention, whereas little is known about the effect of such change on belowground soil carbon pools. In particular, the response of SOC dynamics to addition of elevated CO$_2$-grown plant residues has received much less attention.

Most of the carbon held in terrestrial ecosystems is in the soil and it is important to evaluate the interaction of high CO$_2$ concentrations with decomposition processes and carbon stores. The soil carbon pool is the largest active pool of the global carbon cycle at the Earth’s surface, currently estimated to contain about 1500 Pg C of organic carbon, which is about four times the carbon in biomass and three times the carbon in the atmosphere (Janzen, 2004; Lal, 2004). Soil organic matter (SOM) is known to act both as a source and a sink in global CO$_2$ cycles. Given the size of the soil carbon pool, even small changes in such large pools of carbon would be expected to have dramatic feedbacks in the global climate system. Losses of soil organic matter by soil respiration could contribute to atmospheric CO$_2$ and exacerbate global warming. On the other hand, increases in the storage of SOM could slow the rise of atmospheric CO$_2$ and provide a negative feedback to global warming. However, the response of soil carbon inventories, and particularly of belowground processes (i.e. rhizosphere respiration and
soil organic matter decomposition) to elevated CO$_2$ concentrations is poorly understood.

Sequestration of carbon in soil ecosystems depends not only on total biomass but also on the biochemical properties of the biomass, produced under elevated CO$_2$, in terrestrial system. Many researchers have proposed that the steady increase in atmospheric CO$_2$ concentration is likely to affect biota by producing changes in both plant growth and plant-tissue chemical composition. Among such composition changes, increases in soluble carbohydrates were widely reported. However, demonstrating clear, general effects of elevated CO$_2$ on plant secondary metabolism has proved more difficult. Some studies (Lavola and Julkunentiitto, 1994; Lindroth et al., 1993; Roth and Lindroth, 1994) have shown an increase in the levels of carbon-based secondary compounds, such as phenolics and tannins, while others have not (Fajer et al., 1992; Johnson and Lincoln, 1991; Lincoln and Couvet, 1989). Consequently, decomposition of the plant residues derived from elevated CO$_2$ have shown mixed results. However, all of the research has indicated there is a strong inter-dependence between soil organic carbon decomposition and residue chemical composition. It was reported that residue quality affects both soil microbial community structure and their heterotrophic pathways, which regulate many soil processes. Therefore, examination of changes in residue chemical composition under elevated CO$_2$ as part of the response of plant-soil ecosystem to rising atmospheric CO$_2$ has been urgently needed in global change research.
1.2. Literature Review and Background

1.2.1. Atmospheric [CO$_2$] and Global Change

1.2.1.1. Atmospheric [CO$_2$] History

The historical record of atmospheric CO$_2$, extending to 160,000 Y BP (years before present), is obtained from analysis of gas bubbles trapped in Antarctic ice (Barnola et al., 1987). Until the last century, atmospheric CO$_2$ concentrations oscillated only by approximately 100 $\mu$mol mol$^{-1}$, between 180 and 280 $\mu$mol mol$^{-1}$, with the lowest values found in the layers of ice that were deposited during the last period of continental glaciations (Barnola et al., 1987; Falkowski et al., 2000). Following the rapid glacial melting, the concentration rose to about 260 to 280 $\mu$mol mol$^{-1}$. The pattern of low CO$_2$ during glacial periods every ca.100,000 year interspersed with relatively short, CO$_2$–enriched interglacial periods lasting 15,000 year is known to have persisted for at least the past 25,000 yr and probably extends back 1-3 million years. (Raymo, 1992; Trabalka, 1985). However, since the industrial revolution of the 19$^{th}$ Century, the CO$_2$ concentration rapidly increased from about 280 $\mu$mol mol$^{-1}$ to 370 $\mu$mol mol$^{-1}$ today because of the combustion of fossil fuels and deforestation. Over glacial time scales, it is impossible to tell whether changes in CO$_2$ led to changes in climate, but the association of high CO$_2$ and warm climates is undeniable.

1.2.1.2. “Keeling Curve”

Dr. Charles David Keeling, professor at Scripps Institution of Oceanography, was the first to measure CO$_2$ in the atmosphere on a continuous basis. His measurements started in 1958. Since then, CO$_2$ has steadily risen above the average value of 315 $\mu$mol
mol$^{-1}$ in 1958 when Dr. Keeling started his measurements. By the year 2002 it had risen to about 370 µmol mol$^{-1}$ (Keeling and Whorf, 2002). The oscillating rise of carbon dioxide gas in the atmosphere is known as the "Keeling Curve," named after Dr. Keeling. It is Dr. Keeling who discovered the annual fluctuations of CO$_2$ concentration in atmosphere (the little wiggles in the curve) and he was the first to observe the continuous rise of global atmospheric concentrations of carbon dioxide.

Dr. Keeling deduced that the little wiggles in the Keeling Curve reflect changes in the seasonal storage of carbon in vegetation through photosynthesis and respiration. Every spring when plants start growing, the carbon dioxide in the air decreases, reflecting dominance of uptake from photosynthesis. Conversely, in fall, when leaves and wilted plants are returned to the soil and decay, the carbon dioxide rises again as soil respiration dominates. The oscillation is most pronounced in the Northern Hemisphere, which contains most of the world’s continental area and about two-thirds of the terrestrial vegetation (D'Arrigo et al., 1987). In the Southern Hemisphere, the smaller fluctuations in atmospheric CO$_2$ appear to be driven mainly by exchange with seawater (Keeling et al., 1984). The annual fluctuations of CO$_2$ concentration in the Keeling Curve show that the carbon dioxide concentration changes seasonally over quite a large range of concentrations, indicating the terrestrial ecosystem plays a critical role in regulating atmospheric CO$_2$ concentration.

Comparison of the present atmospheric concentration of CO$_2$ (370 µmol mol$^{-1}$) with the ice core record of pre-Industrial Revolution (< 280 µmol mol$^{-1}$) reveals that atmospheric CO$_2$ is now nearly 100 µmol mol$^{-1}$ larger within a short period of less than 200 yrs, and has rapidly risen at a rate of 1.5 µmol mol$^{-1}$ over the last 45-50 years.
Based on constant per capita fossil-fuel use and population increase, it has been widely accepted that atmospheric CO$_2$ concentration will increase from our present level of 370 µmol mol$^{-1}$ to a level of 550 µmol mol$^{-1}$ CO$_2$ by the middle of the 21st century (Levine, 1992), and to 700 µmol mol$^{-1}$ by the end of the 21st century (Prentice, 2001). Clearly, a continuous increase in atmospheric CO$_2$ concentration is one of the well-documented phenomena of global-scale environmental change (Keeling et al., 1998), and the trend, which seems certain, will continue through this century.

1.2.1.3. Consequences of Rising [CO$_2$]

Rising atmospheric carbon dioxide (CO$_2$) concentration is a concern because of its potential consequences for altering operation of the Earth system. The adverse alterations, which may have already begun include:

1) Warmer air temperature. Global mean surface temperature have increased 0.6°C since the late 19th Century, and by 0.2-0.3°C over the past 40 years. Climate models predict that the mean annual global surface temperature will increase 1-3.5 °C by 2100, with warming more pronounced at higher latitudes (IPCC, 2001).

2) More frequent drought and wildfire. Since 1980, the Earth has experienced 19 of its 20 hottest years on record, with 1998 the hottest and 2002 and 2003 coming in second and third. Dry conditions produced more serious wildfires. In 2002, the Western United States experienced its second worst wildfire season in the last 50 years; more than 7 million acres burned in Colorado, Arizona, and Oregon (Janzen, 2004).

3) More intense rainstorms and flooding. Precipitation has increased about 10% across the contiguous USA since 1910, with a recent increase of one additional heavy
and extreme daily precipitation events every two years (Karl and Knight, 1998). Vermont, New Hampshire, Rhode Island, and Massachusetts each received more than double their normal monthly rainfall in June 1998. Severe flooding in Texas, Montana, and North Dakota during the summer of 2002 caused hundreds of millions of dollars in damage.

(4) Melting glaciers/early ice thaw. Glaciers in the European Alps have lost 30-40% of their surface area and approximately half their volume since the mid-1800s, with an additional loss of 10-20% of their remaining volume since 1980. Since the late 1980s, warming of alpine permafrost indicates acceleration by a factor of five to ten. Melting of ground ice also accelerated markedly from 1980-1990 compared with 1970-1980 (Haenerli and Beniston, 1998). The extent of the ice pack in the Arctic was reduced by 9% in 1990-1995 compared with 1979-1989 (Maslanik, 1996).

(5) Sea-level rise. The global sea level has risen by 10-25 cm over the past 100 years, which corresponds to the low end of model projections of 15-95 cm by the Intergovernmental Panel on Climate Change (IPCC, 1995).

(6) Damage to terrestrial ecosystems. As CO$_2$ increases, it seems probable that at least some species will become extinct, either as a direct result of physiological stress or via interactions with other species. For example, much of the world’s flora has long been adapted to the pre-industrial CO$_2$ level as a baseline [CO$_2$]. Except for rapidly evolving annuals and short-lived perennials, the past century of anthropogenic CO$_2$ enrichment may be an insufficient period for evolutionary adaptation to rising CO$_2$. Even for annual species, this period may be inadequate for CO$_2$-dependent evolution because most of the atmospheric CO$_2$ rise has occurred in the past 50 yrs. Furthermore,
global precipitation patterns may break up, predicted as land-surface precipitation continues to increase at the rate of 0.501% decade$^{-1}$ in much of the Northern Hemisphere especially in mid and high latitudes, and decrease in sub-tropical land areas at the rate of 0.3% decade$^{-1}$ (Hughes and Centre, 2000). This may also decrease the SOC pool and structural stability, increase soil’s susceptibility to water runoff and erosion, and disrupt cycles of water, carbon (C), nitrogen (N), phosphorus (P), sulfur (S) and other elements, and cause adverse impacts on biomass productivity, biodiversity and the environment (Janzen, 2004; Lal, 2004). Of all the alterations, the influence of elevated CO$_2$ on the global carbon cycle, especially on terrestrial carbon cycle, is direct and profound.

1.2.2. Global Carbon Cycling and “Missing Carbon”

1.2.2.1. Carbon Pools

The five principal global C pools of actively cycling carbon are atmospheric CO$_2$, biota, soil organic matter, geologic fossil, and the ocean. Of these, the oceans contain the largest reserves of C, about 39,000 Pg C, though most of this is in deep ocean layers and not in active circulation (Lal et al., 1995). The atmosphere, now with a CO$_2$ concentration of 370 μmol mol$^{-1}$ (Keeling and Whorf, 2002), contains about 785 Pg C as CO$_2$. Carbon stocks in biota are somewhat less certain, but are almost comparable to the atmospheric pool: about 400-600 Pg C (IPCC, 2001; Smil, 2002). The geologic pool, holding about 5000 Pg organic C (Lal, 2004), initially was the most inactive pool, but since Industrial Revolution human beings have been using fossil fuels as energy
sources. Through human use of fuels, the geological pool has become an active pool in the global carbon cycle.

Soil is the largest pool of actively cycling C in terrestrial ecosystems and plays a major role in the global carbon cycle. There are two types of carbon pools in soils: organic (SOC) and inorganic (SIC) pools. The SOC pool is concentrated near the soil surface (within the top 1 m depth), and is estimated at about 1550 Pg in various organic forms, ranging from recent plant litter to charcoal to very old humified compounds (Amundson, 2001; Lal, 2004; 1995). The SIC pool is contained in the deeper layer (below 1 m depth), and is composed of inorganic carbon in the form of calcium carbonate (CaCO$_3$) or caliche. The SIC pool contains as much as 1700 Pg of carbon (Post et al., 1982; Schlesinger, 1991), but this is often assumed not to be in active circulation. Geographically, the SOC concentration ranges from a low in soils of the arid regions to high in soils of the temperate regions, and extremely high in organic or peat soils. Globally, the total soil organic carbon pool is four times the size of the biotic pool and about three times the size of the atmospheric pool.

1.2.2.2. Carbon Fluxes

All of these C pools---the atmosphere, biota, soil, geologic fossil fuel, and ocean---are connected because of fluxes between the pools. The largest exchange occurs between the atmosphere and land plants. The mean residence time for a molecule of CO$_2$ in the atmosphere before it is removed to another reservoir is about 3 years. Atmospheric CO$_2$ enters terrestrial biomass via photosynthesis, at a rate of about 120 Pg C per year (gross primary productivity (GPP)). But about half of the carbon
fixed by land plants (GPP) is respired by the plants themselves, so net primary production (NPP) on land is only about 60 Pg C yr\(^{-1}\). Estimates of the current terrestrial biomass, 560 Pg C, yield a mean residence time of 9 years for carbon in live biomass (Schlesinger, 1991). The NPP is stored at least temporarily in vegetative tissue, but most eventually enters soil upon senescence. At the same time, heterotrophic respiration by soil microorganisms and fire return an amount roughly equivalent to NPP, \(\sim 60\) Pg C per year back to the atmosphere as CO\(_2\). Exchange of CO\(_2\) between the atmosphere and the oceans is about 90 Pg C per year in both directions (Janzen, 2004; Lal, 2004). Some of this occurs by physical processes, involving the CO\(_2\) carbonate equilibrium. But a surprisingly large exchange also occurs via biological processes, though ocean biomass is a mere 3 Pg C. Its NPP almost equals that of all land plants, with a mass of about 600 Pg C (del Giorgio and Duarte, 2002; Janzen, 2004).

1.2.2.3. “Missing Carbon”

The CO\(_2\) fluxes between pools have been naturally in equilibrium for millions of years. However, this balance has recently been disturbed by human activities, mainly through fossil fuel combustion, and land-use change dominated by deforestation. The global C budget for the decade of the 1990s showed that burning fossil fuel and cement production release about \(6.3 \pm 0.4\) Pg C annually, which is one of the most accurately known values in the global carbon cycle. Deforestation of tropical rainforest and other land uses (e.g. conversion of natural to agricultural ecosystems) release about \(1.6 \pm 0.8\) Pg C annually (Houghton et al., 1987). This value is believed by measurements of the \(\delta^{13}C\) in tree rings and ice cores (Leavitt and Long, 1988; Siegenthaler and Oeschger,
1987). Both tree ring and ice core data show a decline in atmospheric $^{13}$C content that is consistent with a reduction in the pool of organic carbon on land. Of 7.9 Pg anthropogenic C, 3.2 ± 0.1 Pg C was measured as increased atmospheric CO$_2$; the remaining C---about 4.7 Pg C---must be absorbed elsewhere. The oceans can account for 2.3 ± 0.8 Pg C of that. But that still leaves another 2.4 ± 1.3 Pg C unaccounted for, which is called “Missing Carbon” or “Missing Sink” (Prentice, 2001). And, by default, this sink is accumulating in the world’s terrestrial plants and soils---so it is also called the “residual terrestrial sink”, but its specific identity has eluded detection (Houghton, 2003; Schimel et al., 2001).

Scientists do not yet agree where (plants or soils) and why this C is accumulating; possible processes include: CO$_2$ fertilization and increased growth of forests, including re-expansion of forests from past disturbances in the northern hemisphere (Adams and Piovesan, 2002; Houghton, 2003). These processes are understandable because all of these trees are C$_3$ plants with a C$_3$ photosynthetic pathway. It is known that photosynthesis of C$_3$ plants, representing more than 95% of all plant species in the world (Houghton et al., 1990), is not saturated with the current CO$_2$ concentrations. Rising atmospheric CO$_2$ will progressively increase the photosynthesis for C$_3$ plants and thus terrestrial NPP. Photosynthesis for C$_4$ plants, which account for about 21% of NPP, theoretically is not sensitive to CO$_2$, but their photosynthesis will not be reduced under elevated CO$_2$. Regardless of C$_3$ or C$_4$ plants, most of all terrestrial NPP are ultimately incorporated into soil as SOM. Therefore, with regard to the Missing Carbon, the real uncertainty is the response of belowground processes to addition of CO$_2$-enriched plant residues. In other words, a big unknown in this global C balance is
the role of the soils, especially with regard to processes involved in carbon emission and sequestration.

1.2.3. C₃, C₄ Pathway and Their Isotopic Discrimination

1.2.3.1. C₃ Pathway

The C₃ photosynthetic pathway is also called the “Calvin cycle”, which was first elucidated in the 1950s by Melvin Calvin, Andrew Benson, and James A. Bassham. The Calvin Cycle is the so-called the C₃ pathway because the plants with C₃ photosynthetic pathway produce a three-carbon compound, 3-phosphoglycerate (3-PGA), as the first stable product in the multistep conversion of CO₂ into carbohydrate. Although 3-PGA is the first product formed in the fixation of CO₂, it does not form directly from three CO₂ molecules. Instead, 3-PGA forms in two concerted steps from the reaction of CO₂ with a five-carbon sugar, ribulose 1, 5-bisphosphate (RubP). The carboxylation of the C₅ sugar produces a C₆ intermediate that is immediately cleaved into two molecules of 3-PGA. The enzyme that catalyzes this reaction is ribulose bisphosphate carboxylase/oxygenase, or Rubisco, one of three enzymes unique to the Calvin cycle. PGA is metabolized through the Calvin cycle to resynthesize ribulose-1, 5-bisphosphate (RubP), the primary acceptor of CO₂. Carbohydrate for storage and post-photosynthetic metabolism is gained by the transfer of intermediates of the Calvin cycle into synthetic pathways, particularly that leading to sucrose, the major carbohydrate exported from photosynthetic cells. Carbon dioxide is a substrate of photosynthesis and is limiting to the rate of photosynthesis in C₃ species at all light levels. In the short term, an increase in atmospheric CO₂ concentration will, in C₃
plants, lead to increased net leaf photosynthetic CO$_2$ uptake both by increasing the velocity of carboxylation of RubP and suppressing photorespiration. Nobel (1991) predicted that for a doubling of atmospheric CO$_2$, transpiration should decrease 30% to 40% for leaves of C$_3$ plants and their rate of photosynthesis should increase approximately 30%.

1.2.3.2. C$_4$ Pathway

In the 1960s, several plant species were identified that, when supplied with $^{14}$CO$_2$, formed large amounts of four-carbon organic acids as the first products of CO$_2$ fixation instead of three-carbon compound, 3-phosphoglycerate. These plants include maize, sorghum, sugarcane, numerous tropical grasses, and some dicotyledonous plants and are called C$_4$ plants. The leaves of these plants demonstrate an unusual anatomy involving two different types of chloroplast-containing cells: mesophyll cells and bundle sheath cells. The mesophyll cells surround the bundle sheath cells, which in turn surround the vascular tissue. The C$_4$ pathway of CO$_2$ fixation is based on a complex interaction between mesophyll and bundle sheath cells. The pathway involves an initial HCO$_3^-$ fixation in the outer mesophyll cells, transfer of an organic acid from mesophyll to bundle sheath cells, and release of CO$_2$ for subsequent refixation in the Calvin cycle. The enzymes unique to the Calvin cycle in C$_4$ plants are located only in the bundle sheath chloroplasts, whereas oxaloacetate is generated from HCO$_3^-$ and phosphoenolpyruvate (PEP) in the cytosol of mesophyll cells.

During C$_4$ photosynthesis, atmospheric CO$_2$ is initially fixed by phosphoenolpyruvate carboxylase (PEPcase) into C$_4$ acids in the outer mesophyll cells.
C₄ acids are then decarboxylated in the inner bundle sheath cells with the subsequent release of CO₂ and its fixation by ribulose-1, 5-bisphosphate carboxylase/oxygenase (Rubisco) (Hatch, 1987). CO₂ released in the bundle sheath is concentrated to 3-10 times that of ambient partial pressure because of the very low bundle sheath cell wall conductance to CO₂ (Brown and Byrd, 1993; Jenkins et al., 1989). Under the high bundle sheath CO₂ concentration, CO₂ assimilation rate is largely saturated and photorespiration suppressed. Consequently, raising CO₂ above the current ambient concentration is expected to have little or no effect on assimilation. However, many recent studies have question this expectation (Ghannoum et al., 2001; Watling et al., 2000).

1.2.3.3. Isotopic Discrimination

Because of the different mechanisms to fix CO₂, C₃ and C₄ plants discriminate against ¹³CO₂ to different degrees. In general, plants using the C₃ pathway for C-assimilation strongly discriminate against carbon-13 (¹³C), and as a result, the δ¹³C value (see explanation for δ¹³C in Chapter 2) for the plant tissues is much lower than atmospheric CO₂. Discrimination of ¹³C during photosynthesis in C₃ plant is caused by fractionation during diffusion through stomatal pores and fractionation by carbon-fixing enzymes, primarily rubisco (ribulose bisphosphate carboxylase/oxy-genase). These effects were quantified by Farquhar and colleagues (1982):

\[ \Delta^{13}C_p = a + (b-a) \times c/c_a \]
Where $c_i/c_a$ is the ratio of intercellular to ambient carbon dioxide (CO$_2$) partial pressure, $a$ is the fractionation associated with diffusion (4.4 parts per mil [%o]), and $b$ is the net enzymatic fractionation associated with carboxylation. Therefore, discrimination is a function of $c_i/c_a$, which is sensitive to a variety of factors that influence the balance of stomatal conductance and assimilation rate. Usually, the $\delta^{13}C_{\text{PDB}}$ value in C$_3$ plants ranges between -32‰ to -22‰ with an overall mean of -27‰ (Boutton, 1991).

Alternatively, plants using the C$_4$ pathway do not discriminate as strongly as C$_3$ plants, and the $^{13}C/^{12}C$ ratios of the plants are closer to that of atmospheric CO$_2$. In C$_4$ plants, carbon is first fixed by phosphoenolpyruvate (PEP) carboxylase in the mesophyll and then transported into bundle sheath cells. In these cells, rubisco is physically isolated from the stomatal cavity. There is no fractionation associated with a product if all of the substrate is utilized. Therefore, the only consideration is the fractionation factor for rubisco to the extent that CO$_2$ or HCO$_3^-$ (hydrocarbonate) leaks out of bundle sheath cells back into the stomatal cavity. These effects were quantified by Farquhar (1983).

$$\Delta^{13}C_p = a + (b_4 + b_3\phi - a) \times c_i/c_a$$

Where $b_4$ is the fractionation associated with PEP carboxylase (-5.7 %o), $b_3$ is the fractionation associated with rubisco (30%), and $\phi$ is the leakiness of the bundle sheath. For many species, the term $(b_4 + b_3\phi - a)$ is close to zero, such that $\Delta^{13}C$ may show little environmental variation in C$_4$ plants (Farquhar et al., 1989). As a result, C$_4$ plants have
a $\delta^{13}C_{PDM}$ value in the range of -17‰ to -9‰ with an overall mean of -13‰ (Boutton, 1991).

1.2.4. Plant-Soil Ecosystem Response to Elevated CO$_2$

1.2.4.1. Plant Growth

Since 1977 when the US Department of Energy (DOE) sponsored a workshop in Miami, Florida, which first officially incorporated plants and terrestrial ecosystems into its Carbon Dioxide Research Program (Luo et al., 1999), impacts of elevated CO$_2$ on plant growth have received considerable attention because CO$_2$ is not only a major greenhouse gas, but also a substrate for photosynthesis of plants. Over two decades of studies, researchers using CO$_2$ experiments have presented more evidence to support the hypothesis that the growth or biomass of plants increases when grown under elevated CO$_2$---the so-called “direct CO$_2$-fertilisation effect” (Curtis and Wang, 1998; Mooney et al., 1999).

Theoretically, the photosynthesis of C$_3$ plants is not saturated at the present atmospheric CO$_2$ concentration. The principal carbon-fixing enzyme in plants is ribulose 1,5-bisphosphate carboxylase/oxyylase (rubisco). In C$_3$ plants, the activity of rubisco increases with increasing CO$_2$ concentration as a consequence of reduced competitive inhibition by O$_2$ (Bazzaz, 1990). Consequently, as atmospheric CO$_2$ increases, elevated CO$_2$ levels will lead to an enhanced initial CO$_2$ assimilatory reaction, resulting in increased growth and biomass accumulation. Researchers have broadly reported that plants, especially C$_3$ plants, exposed to elevated CO$_2$ often show increased growth (Rogers and Dahlman, 1993). Poorter (1993) surveyed the literature
(156 plant species) and found the average stimulation of vegetative whole plant growth to be 37%. Kimball (1983) reported that doubling the CO₂ concentration increases CO₂ assimilation rate of C₃ plants and this generally explains the 30-44% increase in plant dry mass in response to high CO₂ concentration. Generally, the controlled environment and field studies over the last 30 years have verified the enhancement of C₃ plant growth in response to elevated CO₂.

In contrast to the study of C₃ plants, whether rising atmospheric CO₂ concentration will also increase productivity of C₄ plants is still uncertain. The reason for this uncertainty is that relatively few CO₂ enrichment studies have focused on the response of C₄ plants to high CO₂ compared to the large number of studies on C₃ plants (Ghannoum et al., 2000), and also because C₄ plants are physiologically less responsive to elevated CO₂. C₄ plants have a specialized leaf anatomy and biochemistry that results in a three- to ten-fold higher concentration of CO₂ in the bundle sheath cells compared to that in C₃ plants (Furbank et al., 1989). C₄ plants are biochemically adapted to reduce photorespiration through mechanisms that effectively increase the CO₂ concentration presented to the rubisco enzyme (Hatch, 1992). Unlike C₃ species, the rate of net C-assimilation for C₄ plants is generally not limited by CO₂ supply, and consequently, C₄ plants were not expected to respond to rising atmospheric CO₂ (Johnson et al., 1993; Nobel, 1991). Maize and sorghum are the two main C₄ crops, and experiments showed that they generally tend to have little or no growth response to elevated CO₂ under well-watered conditions (Carter and Peterson, 1983; Cure and Acock, 1986; Morison and Gifford, 1984; Ottman et al., 2001; Patterson and Flint, 1980). Based on these early results and the known CO₂ concentration mechanism of C₄ photosynthesis, it was
generally assumed that C₄ plants would not respond to elevated CO₂ under well-watered condition.

However, more recent studies have found that the growth of many C₄ plants increased in response to elevated CO₂ even under well-watered conditions (Ghannoum and Conroy, 1998; Wand et al., 1999; Watling et al., 2000; Ziska et al., 1999). It was recently reported that, on average, the growth stimulation of C₄ plants in response to a doubling of the current ambient CO₂ is about 22-33%, compared with 30-44% for C₃ plants (Poorter, 1993; Wand et al., 1999). Furthermore, it was reported that the growth stimulation of C₄ weeds is larger than that of C₄ crops (Poorter, 1993; Ziska and Bunce, 1997). Ghannoum et al. (2000) proposed several possible mechanisms that elevated CO₂ enhances the growth of C₄ plants: including by raising the intercellular CO₂ concentration and consequently CO₂ assimilation rate; and by reducing bundle sheath cell wall conductance to CO₂ and consequently leaf transpiration rate; by reducing mitochondrial respiration and consequently whole plant respiratory losses, which can contribute to biomass increases.

Recently, the growth analysis approach separates plant growth rate into several components, including net assimilation rate per unit of leaf area, leaf area ratio, specific leaf area, and root:shoot ratio. These components have been used to further assess the impact of global change on plant growth and allocation of carbon assimilates. The review summarized by Pritchard et al. (1999) showed that plants grown in elevated CO₂ exhibited increased leaf area per plant in 66% of studies, whereas 28% of observations reported no change and 6% reported a decrease in whole plant leaf area. This resulted in an average net increase in leaf area per plant of 24%. Underground root growth in
response to elevated CO₂ has direct special effects on soil microbial processes, including SOC humification and decomposition. Poorter (1993) noted that elevated CO₂, in addition to increasing whole plant biomass, altered root:shoot ratios. Rogers et al. (1997) reviewed the available literature for crop species and found that 60% of articles showed an increase in root:shoot ratios under elevated CO₂. This increase is almost entirely attributed to fine root production. The most pronounced effects were increases in root length (110%) and root dry weight (100-200%) (Idso and Kimball, 1992; Rogers et al., 1992; Stulen and den Hertog, 1993). The proportion of carbohydrate that is assimilated and transferred to the roots is critical in understanding SOM dynamics under elevated CO₂ conditions.

It should be pointed out that, during more than two decades of study on the effects of CO₂ enrichment on plants, almost all of the initial short-term experiments demonstrated that elevated CO₂ concentration alleviated the limitation of C₃ photosynthesis by CO₂ supply, but subsequent and often longer-term experiments have shown that photosynthesis could acclimate downwards in response to CO₂ enrichment (Adam et al., 2000; Woodward, 2002). The reason for this photosynthesis down regulation is in part because of soil nitrogen (N) availability decreased by SOC slower decomposition---so called the “litter quality” hypothesis (the litter quality hypothesis states that litter N concentration is lower in CO₂-enriched plants and its decomposition rate reduced. This, in turn, would result in lower N availability in the soil, providing a negative feedback to photosynthesis and plant growth). Alternately, there is now some evidence to suggest that photosynthesis is largely stimulated in C₄ species in response to long CO₂ enrichment (Ghannoum et al., 2000), and that the land area occupied by C₄
grasslands is likely to increase under the global warming scenario for the 21st Century (Archer, 1993; Henderson et al., 1994).

1.2.4.2. Plant Chemistry

The possibility that CO$_2$ enrichment will alter the chemistry of litter was first proposed by Strain and Bazzaz (1983). At that time there was evidence suggesting that the foliar nitrogen (N) concentration was lower in CO$_2$-enriched plants, so it was reasonable to assume that the N concentration in leaf litter would also be lower. Since then, a number of studies have evaluated the effect of elevated CO$_2$ on litter chemistry, most of which focused on N or C:N ratio of litter. That elevated CO$_2$ tends to decrease N content, leading to an increased C:N ratio of plant litter, has been widely supported (Mooney et al., 1991). Several compilations of the literature have reported declines in foliar [N] in tree species averaging 21% (McGuire et al., 1995), 16% (Curtis and Wang, 1998), 14% (Cotrufo et al., 1998), or 13% (Norby et al., 1999), with a concomitant increase in C:N ratio. Meanwhile, researchers also reported that elevated CO$_2$ increases litter total non-structural carbohydrate (TNC) by 13% in *Myrtus communis* (Peñuelas et al., 2002), 16.8% in *B. erectus* and 29.4% in *D. glomerata* (Roumet et al., 1999).

Melillo (1983) suggested that high TNC and lower N concentration in the litter produced at high CO$_2$ would be because of increased carbon assimilation relative to nitrogen assimilation.

However, providing a clear statement about the changes in secondary metabolism, like phenolics and terpenes, and secondary carbon-based structural compounds, like cellulose, hemicellulose, lignin and pectin, of plant litter in response to
elevated CO₂ has come to be more difficult. This is because the studies on the effect of elevated CO₂ on these C-based secondary compounds (CBSC) are few in number and also because the effect of elevated CO₂ on plant CBSC depends on plant species and interactions with other environmental factors (e.g., water and nutrient availability, temperature). Norby et al. (2001) summarized 46 experimental observations and found lignin concentration in leaf litter ranged 23-253 mg g⁻¹ with a median of 138 mg g⁻¹ in ambient CO₂ and ranged 26-287 mg g⁻¹ with a median of 149 mg g⁻¹ in elevated CO₂. Meta-analysis of the lignin data indicated a significant effect of CO₂ treatment, with a 6.5% increase in elevated CO₂ (Curtis and Wang, 1998). Van Ginkel et al. (1996) reported that grass root material grown at lower CO₂ had a higher cellulose fraction than roots grown at elevated CO₂ concentration, but the lignin fraction of the roots grown at elevated CO₂ was higher than those grown at 350 µmol mol⁻¹ CO₂. Booker et al. (2000) reported that lignin concentrations in leaves and stems of cotton were not significantly affected by elevated CO₂ (722 µmol mol⁻¹), whereas root residue lignin concentration was 30% greater than the cotton treated with ambient CO₂. Peñuelas et al. (2002) reported that phenolic concentrations in Erica arborea foliage were 28% lower under high CO₂ condition near a CO₂ spring site than in the low [CO₂] control site. Reports on specific CBSC compounds of CO₂-enriched plants are difficult to find.

Because levels of CBSC are affected by environmental conditions, resource allocation hypotheses such as carbon-nutrient balance (CNB) (Bryant et al., 1983) and growth differentiation balance (GDB) (Herms and Mattson, 1992) have been proposed to predict the effect of environmental factors on plant CBSC. The CNB hypothesis predicts that plants growing under elevated CO₂ have an ‘excess’ of carbon relative to
the nutrients available for growth, and use it to produce terpenes, phenolics and other
carbon-based compounds, and the GDB hypothesis assumes that the consequent rise of
the carbon-nutrient availability ratio under elevated CO$_2$ limits growth more than
photosynthesis, which promotes a relative increase of carbon availability that exceeds
growth requirements and results in an accumulation of TNC and CBSCs. Both of these
hypotheses predict that elevated CO$_2$ will increase the CBSCs of the plants.

As described in both CNB and GDB hypotheses, impact of elevated CO$_2$ on
plant chemistry was triggered by the difference of carbon and nitrogen availability. C$_4$
plants have a different response to C and N availability from C$_3$. At the current CO$_2$
concentration and a given N supply, C$_4$ plants have higher nitrogen-use efficiency
(NUE) than C$_3$ plants. The higher NUE results from a lower investment of leaf N in
rubisco protein compared to C$_3$ plants (Brown, 1978; Pheloung and Brady, 1979). For
C$_4$ plants, there are conflicting results in the literature regarding the influence of high
CO$_2$ concentration on N concentration in leaves of C$_4$ plants. Reduced foliar N
concentration is reported in some studies (Morgan et al., 1994; Owensby et al., 1994;
Read and Morgan, 1996), but not in others (Ghannoum and Conroy, 1998; Ghannoum
et al., 1997). Although the growth of C$_4$ plants is less responsive to elevated CO$_2$
(Hatch, 1992), elevated CO$_2$ has been reported to cause modifications in the C$_4$
pathway at anatomical and metabolic levels, as well as other physiological processes.
Metabolic changes included a decrease in carboxylation efficiency and the CO$_2$
saturated rate of photosynthesis. Anatomical changes included a two-fold decrease in
bundle sheath cell wall thickness, which increased bundle sheath leakiness (Watling et
al., 2000). Physiological process changes include an increase in net assimilation rate
(Wand et al., 1999; Ziska et al., 1999), a reduction in stomatal conduction and an increase in water–use efficiency (Wall et al., 2001). Any of these changes may cause the imbalance of C and N supply in C$_4$ plant and the modification in the plant biochemical composition.

It was also reported that chemical changes of plants in response to elevated CO$_2$ vary during plant growing stage. Larigauderie et al. (1988) observed that, in their CO$_2$ experiment with the grass *Bromus mothis*, leaves of the grass had a higher C:N mass ratio for the first 2 months of growth in the elevated CO$_2$, but that the CO$_2$ effect was not present in mature leaves at day 130. Similarly, for the grapes studied by Bindi et al. (2001), substantial CO$_2$ effects on grape acid and sugar concentrations in the middle stages of the growth season were reduced after the plant matured. Roth et al. (1998) reported that enriched CO$_2$ significantly increased non-structural carbohydrates in expanding aspen leaves, but that the difference disappeared upon leaf maturation. If the response of plant chemical composition to elevated CO$_2$ is moderated by plant phenology, the changes in chemical composition of matured litter are more important in studying new organic decomposition and soil carbon sequestration.

1.2.4.3. Soil Respiration

Soil respiration (SR) is usually understood as the evolution of biologically generated CO$_2$ from the soil surface to the atmosphere. It is expressed as a flux (e.g. gCO$_2$ [or C] m$^{-2}$ d$^{-1}$). Although precise estimates of carbon recycled to the atmosphere from belowground sources are unavailable, Musselman and Fox (1991) propose that the belowground contribution exceeds 100 Pg y$^{-1}$ globally. This represents a major
component of C flux in the global C cycle. Currently, impacts of elevated CO\textsubscript{2} on soil respiration are unknown. Results from field studies have shown that soil respiration response to elevated [CO\textsubscript{2}] varies among ecosystems. A significant increase in soil respiration (50\%) was detected in a mid-successional lowland forest soil exposed to 693 \, \mu mol \, mol^{-1} [CO\textsubscript{2}] compared to 352 \, \mu mol \, mol^{-1} [CO\textsubscript{2}] (Ball et al., 2000). Seasonal average respiration of a sandstone grassland at 500 \, \mu mol \, mol^{-1} CO\textsubscript{2} increased 42\% compared to the ambient CO\textsubscript{2} treatment (Luo et al., 1999). In our prior FACE experiment with cotton and wheat at the Maricopa Agricultural Center, Arizona, CO\textsubscript{2} enrichment significantly increased soil respiration (Nakayama et al., 1994; Pendall et al., 2001). However, soil respiration rate in the Alaskan tundra was not significantly affected by either long- or short-term CO\textsubscript{2} enrichment (Oberbauer et al., 1986). In a Populus forest, soil respiration was not affected by doubling atmospheric CO\textsubscript{2} (Randlett et al., 1996). Variations in soil respiration rates under elevated CO\textsubscript{2} may be associated with other ecosystem attributes and processes.

The sources of CO\textsubscript{2} from soil respiration include live root respiration, litter (e.g. exudates, sloughed-off material, dead roots and other dead plant tissues) decomposition and soil humus breakdown (Buyanovsky and Wagner, 1995). The respired CO\textsubscript{2} coming from these different sources will determine the C storage potential in the soil. Generally, more CO\textsubscript{2} derived from root-related activity (e.g. root metabolism and heterotrophic decomposition of exudates, sloughed-off material, and dead roots) indicates higher plant growth and more organic C input to the soil, whereas more CO\textsubscript{2} coming from soil organic carbon (SOC) breakdown will result in more SOC loss and
the final reduction of soil C storage (Lambers et al., 1996). Obviously, it is equally
critical for estimating feedback between global change and soil processes to study the
influence of CO$_2$ enhancement on total soil respiration and the contribution of CO$_2$
sources to the total soil respiration as well.

However, compared to the number of papers on bulk soil respiration response
to elevated CO$_2$, very little research has been done on partitioning this respiration. The
main obstacle is that with current methods, it has proven difficult to determine the
relative contribution of various sources to total soil CO$_2$ fluxes measured in the field,
especially the CO$_2$ contribution from root respiration and decomposition of newly-
formed plant matter, which are distinguished with difficulty (Pendall et al., 2001).
Elevated CO$_2$ has been widely reported to increase plant root growth, which has the
potential to increase root respiration (Farrar, 1981). Enhanced root growth could
promote more carbon exudation resulting in elevated microbial activity in the
rhizosphere (increased mycorrhizal infection and increased N-fixation) (Diaz et al.,
1993; Zak et al., 1993). It was reported that microbial carbon was 1.5 times greater in
the rhizosphere of plants grown under elevated CO$_2$ compared with plants grown under
ambient CO$_2$ (Diaz et al., 1993; Zak et al., 1993). Körmer and Arnone (1992) and Diaz
et al. (1993) both provide evidence that under elevated CO$_2$ carbon exudation and
microbial activity in the rhizosphere were enhanced. The relative contribution of roots
versus other soil components has been estimated to vary between 35 to 65% of the total
CO$_2$ evolved (Ewel et al., 1987).
1.2.4.4. SOC Decomposition

Direct effects of rising atmospheric CO\textsubscript{2} levels on SOC decomposition are unlikely to be important because of the inherently high soil concentration of CO\textsubscript{2} (van Veen et al., 1991). However, changes in the decomposition rates have been predicted because the enrichment of atmospheric CO\textsubscript{2} can substantially modify the soil environment, by increasing belowground C allocation, and changing water and nutrient availability, and modify the quality of the plant residues. Carbon input to soil generally increases under elevated CO\textsubscript{2} condition owing to improved plant carbohydrate status for C\textsubscript{3} plants and even for less-responsive C\textsubscript{4} plants (Körner and Arnone, 1992).

Quantitative alterations in C inputs to the soil include enhanced rhizodeposition and root exudates, increased C supply to symbionts (mycorrhizae and rhizobium) and increased total litter inputs (Paterson, 1997; Rouhier, 1994; Soussana and Hartwig, 1996; Zak et al., 1993). Because soil microorganisms are commonly C-limited, increased C availability, resulting from increased [CO\textsubscript{2}], generally stimulates microbial growth and activity. In general, compared with old soil organic matter decomposition, newly added plant materials are rapidly degraded because the acquisition of energy for microorganism activities from soil organic carbon substrate is lower than fresh organic carbon. However, researchers have reported litter of plants grown in high CO\textsubscript{2} environments often have higher C:N or lignin:N ratio, C-based secondary compounds such as phenolics and tannins, and structural compounds such as cellulose and lignin (Cotrufo and Ineson, 1996; Gebauer et al., 1998; Peñuelas and Estiarte, 1998). It is commonly believed that the low quality of organic matter limits the amount of available energy for soil microorganisms, and in turn the rate of soil organic matter
decomposition (mineralization). Whether or not higher inputs of organic matter will increase the content of soil organic matter (SOM) depends largely on organic material quality (Van Ginkel et al., 1996).

CO2 enrichment often causes reductions in stomatal conductance and stomatal density, leading to increased water-use efficiency (WUE). Improved WUE can significantly influence soil microbes in water-limited environments by increasing soil water availability and indirectly, through lengthening the period of plant growth. Therefore, water limitation of microbial activities can be alleviated and the period of active microbial decomposition can be lengthened in arid and semi-arid ecosystems. In addition to carbon and water sources, microbial activity also needs other nutrients, in competition with plants. It was reported that CO2-enhanced plant growth is often accompanied by increased plant nutrient uptake (Gorissen, 1996; Hodge, 1996). Concentrations of available nutrients in soil are usually lower in plots with elevated CO2. Although nutrient competition between plant and soil microbes is expected, less is known about the relative competitive ability of plants and microbes for nutrients during CO2 enrichment. Elevated CO2 might increase plant nutrient uptake by enhancing production of fine roots, nutrient uptake rates and mycorrhizal infection or activities, thus altering the dynamic equilibrium between plant and microbes in N acquisition (Jackson and Reynolds, 1996; Klironomos et al., 1996). Improved soil moisture stimulates microbial growth in water-limited systems, whereas nutrient limitation to soil microbes may reduce the microbial activity, and thus SOC decomposition.

Although elevated atmospheric CO2 alters C, water and nutrient status in the soil, the consequences of altered SOC decomposition are not clear, owing to the
complex and dynamic interactions between plant, soil and microbes. Previous research on decomposition rate of SOC under elevated CO\textsubscript{2} has been contradictory. For example, King et al. (2001) found that decomposition rate of \textit{Populus tremuloides} litter from open-top chambers did not differ between ambient and elevated CO\textsubscript{2} conditions. Others have observed decreased rates of litter decomposition resulting from exposure of plants to elevated CO\textsubscript{2} (Ball, 1997; Cotrufo and Ineson, 1996). In a field experiment with litter bags, Sowerby et al. (2000) showed a positive effect of elevated CO\textsubscript{2} on decomposition and reported that \textit{L. perenne} litter originally grown in elevated CO\textsubscript{2} consistently had a greater rate of decomposition than the litter grown in ambient CO\textsubscript{2}. In most cases, Klironomo et al. (1996) summarized that when experiments are performed in the absence of living plants, plant materials grown under elevated CO\textsubscript{2} often decompose more slowly than those produced at ambient CO\textsubscript{2}. However, there are insufficient studies on litter decomposition to generalize about certain CO\textsubscript{2} effects on decomposition in both managed and natural ecosystems.

1.2.4.5. SOC Storage

Soil organic carbon (SOC) storage is determined by the balance of the input of C from above- and belowground-plant residues and the output from the mineralization of SOM, released as CO\textsubscript{2} (and as CH\textsubscript{4} in anaerobic soils). In a steady-state situation, these inputs are equal to the NPP of a system, which is largely controlled by climate with some influence of soil properties such as fertility status, soil texture and moisture. Under given conditions, elevated CO\textsubscript{2} has been widely reported to increase NPP, but this alone cannot answer the question of whether or not elevated CO\textsubscript{2} will increase SOC.
storage, because belowground soil processes are still unknown. Newly incorporated plant residues in soil can either stimulate or retard decomposition of native SOC.

Currently, there are two hypotheses regarding elevated CO\(_2\) effects on SOC storage. The “Priming Effect” hypothesis states that increased soil microbial activity caused by additional C entering soil in an elevated-CO\(_2\) environment would lead to increased decomposition rate of soil organic matter, and consequently reduce total soil organic carbon storage. This hypothesis is based on the concept that soil microorganisms are commonly C-limited, and increasing fresh organic C input resulting from elevated CO\(_2\) would stimulate microbial growth and activity because of the higher availability of energy released from the decomposition of this fresh organic matter (Fontaine et al., 2003). The “Priming Effect” hypothesis predicts that although elevated CO\(_2\) increases plant residue input to soil, total SOC storage would decrease. The “Priming Effect” viewpoint originated at the beginning of 20\(^{th}\) Century. One of the first investigators to suggest a priming action by addition of organic materials to soil was Löhnis (1926). From results of “green manuring” experiments in the field and green-house, he concluded that intensified bacterial activity accompanying incorporation of immature plant residues in soil increased mineralization of the native humus N. This hypothesis has been challenged from time to time and has yet to be resolved.

In contrast, the “Litter Quality” hypothesis suggests that carbon storage in soil will increase under an elevated atmospheric CO\(_2\) concentration because of a combination of an increased net CO\(_2\) uptake, a shift in carbon allocation pattern in the plant/soil system, a decreased decomposition rate of low-quality plant residues, and the
consequence of retarded native humus decomposition. The “Litter Quality” hypothesis emphasizes plant chemistry effects on decomposition and elevated CO$_2$ effects on residue quality. The chemical composition of CO$_2$-enriched plant has been reported by many researchers to regulate residue decomposition, but which chemical compounds are the most related to decomposition still is in question. For example, Melillo et al. (1982) reported that litter decomposition rate was related to its initial N concentration, lignin concentration, C:N ratio, and lignin:N ratio. Tian et al. (1992) reported that the most important chemical characteristics influencing litter decomposition are C:N ratio, lignin and polyphenols. Furthermore, Rastetter et al. (1992) pointed out that the C:N ratio of plant tissues is one of the key biogeochemical determinants regulating the amount of C that can be sequestered from the atmosphere into the soil system, whereas Scholes et al. (1997) said that changes in lignin, tannin and polyphenol levels are more important in the decomposability of litter than changes in the C:N ratios. To date, despite an increasing number of studies on the relationship between residue quantity/quality and soil processes (Canadell et al., 1996), little is known about the overall impact of elevated atmospheric CO$_2$ on net ecosystem C storage.

1.2.5. Interaction of CO$_2$ with Water Deficiency

The effects of elevated atmospheric CO$_2$ on terrestrial ecosystems will not occur in isolation, but in the context of multiple environmental changes such as soil moisture, salinity, nutrients, temperature, UV-B, ozone, and atmospheric pollutants. Among all of these factors, the interaction of CO$_2$ with water deficit is probably the most important in plant growth and productivity, as well as to soil microbial growth and
activity, especially in dry areas where water availability limits productivity more than availability of other nutrients. Elevated CO$_2$ tends to ameliorate the effects of water stress and has been shown to increase water-use efficiency for both C$_3$ and C$_4$ plants through decreased stomatal conductance and transpiration rates (Kimball et al., 1993; Lawlor and Mitchell, 1991; Tyree and Alexander, 1993). From a two-year FACE grassland study, with a 192-ppm enrichment, Lee et al. (2001) demonstrated a 23% reduction in stomatal conductance for 13 perennial species, which led to a 40% increase in instantaneous WUE. Nobel (1991) predicted that as atmospheric CO$_2$ levels become twice as high as present level in the latter half of the 21st Century, the WUE should increase about 35% for C$_4$ plants and 75% for C$_3$ plants. The higher WUE is necessary to improve soil moisture conditions that will influence soil nutrient availability and soil microbial activity. Research findings also show that increases in water-use efficiency with elevated CO$_2$ are generally greatest in water-stressed systems (Lee et al., 2001; Nobel, 1991). This implies that water deficit enhances the effects of elevated CO$_2$ on plant-soil ecosystem, and in other words, the impact of elevated CO$_2$ on C dynamics is more prominent for dry areas than for other ecosystems.

1.3. Dissertation Format

The present dissertation consists of three major parts in the formation based on the requirement by the University of Arizona Graduate College: Chapter 1---Introduction, Chapter 2---Present Study, and Appendixes. In the Introduction, there are two sections: The first section describes the research problem and origin, introducing a major problem that the dissertation is intended to address, and the origin of the
problem. In this section, I discuss the possible resolution of the problem based on 6 hypotheses. The second section is literature review and background knowledge. In this section, I summarize the achievements and short-comings of past and present research conducted by other scientists on the problem that I am addressing, and I review some of the related background knowledge. In the Present Study, I list and describe the methods that I used in this study, and the results of my research. Four papers resulting from my research are presented as Appendixes (Appendix A, B, C and D).

The first paper (Appendix A) is about elevated CO₂ effects on soil respiration and soil air CO₂. Dr. Leavitt and Mr. Jim collected the soil respiration data and CO₂ air samples at the easily growth season of 1998 (then I jointed with them) for my first paper from the Arizona Maricopa FACE field at the weekly intervals during the 1998 and 1999 growing seasons. CO₂ air samples were purified and analyzed in the Laboratory of Tree-Ring Research with the help of Nicole Ricketts and Vada Maryol. I finished all of the data calculations and analyses, and the manuscript.

The second paper (Appendix B) provides a detailed analysis of sorghum tissue biochemical composition in response to elevated CO₂. I systematically analyzed the major chemical composition from every soluble carbohydrate to C-based secondary compound in sorghum leaf, stem, and root. Sorghum samples were collected from Arizona Maricopa FACE field at the end of 1999 growing season. Except for lignin analysis that was done in Tree-Ring Lab on the UA campus, all of other chemical composition analyses were done in Dr. Dean Martens’ laboratory in the USDA-ARS Southwest Watershed Research Center at 2000 E. Allen Road, Tucson. I finished all of the data calculations and analyses, and the manuscript.
The third paper (Appendix C) considers the degradability of sorghum plant tissues to address the impact of elevated CO$_2$ on SOC decomposition rate and how tissue chemical composition regulates the residue decomposition. The experiment used a laboratory incubation method. I conducted the experiment in Dr. Dean Martens’ laboratory. Soil and sorghum tissue samples used in this study were collected from Arizona Maricopa FACE experiment. I calculated the correlation between respired CO$_2$ released from incubated soil with tissue chemical compounds, and also did analysis of statistical significance. I wrote the manuscript.

The last paper (Appendix D) describes the impact of elevated CO$_2$ on labile and recalcitrant soil carbon pools. The experiment was conducted in Tree-Ring Lab. In this study, I used the 6N HCl hydrolysis method that I modified successfully to separate the FACE soil organic matter into two C pools. Furthermore, I isolated new input C from original (old) C from each C pool based on the carbon isotopic mixing model, and also calculated the mean residence time (MRT) of bulk SOC with the single exponential decay model. Correlations between the proportions of recalcitrant C in total SOC and the MRT of bulk SOC are considered to confirm the validity of the 6N HCl method. I did all of the lab work and data analyses, and wrote the manuscript. This paper has been submitted to the Journal: Soil Biology and Biochemistry.

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2. CHAPTER II: PRESENT STUDY

2.1. Methods

2.1.1. Field Methods

2.1.1.1. Free-Air CO₂ Enrichment (FACE) Experiment

A sorghum [Sorghum bicolor (L.) Möench] Free-Air CO₂ Enrichment (FACE) experiment was conducted at the University of Arizona Maricopa Agricultural Farm (elevation: 358 meters, 33.1 °N, 112.0 °W), AZ, USA, continuously for two growing seasons in 1998 and 1999. The detailed techniques of the FACE experiment have been given by Ottman et al. (2001), Conley et al. (2001), Cousins et el. (2001), Wall et al. (2001) and Leavitt et al. (in revision). A summary is as follows:

\[ \text{a. CO}_2 \text{ Treatment} \]

The FACE experiment consisted of eight 25-m-diameter plenum rings (2 CO₂ levels × 4 replicates), distributed in a 12-ha sorghum field and separated from adjacent arrays by about 100 m to minimize likelihood of CO₂ contamination (Fig.2-a and 2-b). These 8 rings were constructed from 0.305-m inside diameter PVC (polyvinyl chloride) pipe around which were installed 2.5-m-high vertical stand pipes spaced about every 2.4 m. The rings were placed in the field shortly after planting (Fig. 2-a, -b and Fig. 3-a, -b). In the four enriched (“FACE”) CO₂ plots, air and pure CO₂ were mixed in the PVC pipes and injected through holes in vertical standpipes onto the plots to maintain CO₂ concentrations at canopy level at an average of 190-200 μmol mol-1 above background concentrations measured in the other four ambient (“Control”) CO₂ plots. This blower system was controlled by a computer system, which regulated the CO₂ concentrations
Fig. 2-a, -b. Sorghum (*Sorghum bicolor* (L.) Mönch) Free-Air CO$_2$ Enrichment (FACE) experiment conducted during 1998 and 1999 growth seasons at the University of Arizona, Maricopa Agricultural Center, Maricopa, Arizona (33.1°N, 112.0°W).

There are eight 25m-diameter rings (2 CO$_2$ levels × 4 replicates) (Fig. 2-a), equipped with identical blower system (Fig. 2-b) and separated from adjacent arrays by about 100 m. Of 8 rings, 4 rings exposed sorghum at elevated CO$_2$ (560 µmol mol$^{-1}$) as “FACE”, and the other 4 rings received ambient CO$_2$ (360 µmol mol$^{-1}$) as “Control”. Each ring was split in semicircular halves, with each half receiving either a well-watered (“Wet”) or a water-stressed (“Dry”) irrigation regime. (The photos are from USDA Water Lab in Phoenix, AZ)
within FACE plots based on wind speed, wind direction and CO\(_2\) concentration inside the ring. Identical blower systems with ambient air were installed in the control plots to avoid inducing differences related to air circulation. CO\(_2\) fumigation was applied continuously from the date when 50% of the sorghum plants emerged until plant maturity, 24 hours per day. Average daytime CO\(_2\) concentrations monitored in the center of each array at 10 cm above the crop canopy during the 1998 and 1999 growing seasons were 556 and 566, and 364 and 373 µmol mol\(^{-1}\) in FACE and Control plots, respectively. Average nighttime CO\(_2\) concentrations were slightly higher at 603 and 607, and 428 and 433 µmol mol\(^{-1}\) at FACE and Control plots, respectively. The target CO\(_2\) level set in FACE plots was an atmospheric CO\(_2\) concentration likely to be reached by the late 21\(^{st}\) Century, as projected by IPCC (2001).

\(b. \quad H_2O \text{ Treatment} \)

Each of the circular FACE and Control plots was also split into semicircular halves, with each half receiving either a well-watered (Wet) or a water-limited (Dry) regime (Fig. 2-a, and -b). Maintaining the desired moisture regime level was done by level-basin flood irrigation, which was utilized to maintain soil-water content near field capacity in Wet plots. After 30\% of the available water in the rooted zone at the Wet plot was depleted, the field was irrigated with an amount calculated to replace 100\% of the potential evapotranspiration since the last irrigation. Only two irrigations were applied to dry treatments (post-planting and mid-season) each season compared to 7 (1998) or 5 (1999) in the wet treatments. The total water received by the Wet side was
Fig. 3-a, -b. FACE field layout for the 1998 and 1999 sorghum experiments (Fig. 3-a) and the locations of soil organic carbon (SOC) study plots in Wet and Dry sides (Fig. 3-b). (The figures are provided by USDA Water Lab in Phoenix, AZ)
1218 and 1047 mm of irrigation + rain applied during the 1998 and 1999 growing seasons, respectively, which was slightly more than twice the amount of water received at the dry side (474 and 491 mm during the 1998 and 1999 growing seasons, respectively).

c. Soil in the Site

The soil in the FACE site is classified as a Trix clay loam: fine-loamy mixed (calcareous), hyperthermic Typic Torrifluvents (Kimball et al., 1992; Post et al., 1988), 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 ranging from 30 to 100 cm has similar characteristics to 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, with Core-Method). 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) over several decades as farmland. SOC contents range from about 0.7% in the surface horizon to 0.2% at 100-cm depth. Average soil pH is approximately 7.6 in the entire soil profile.

d. Crop Culture

Sorghum seed (Sorghum bicolor cv. Dekalb DK54) treated with fungicide was planted in north-south rows spaced 0.76 m (30 inches) apart at a rate of 318000 seeds
ha\(^{-1}\) (41 mm apart for 32 seeds m\(^{-2}\); plant density of 21 plants m\(^{-2}\)) on 15-16 July in 1998 and on 14-15 June in 1999, one month earlier in 1999 than 1998. Heading and anthesis occurred from 27 September to 2 October in 1998 and from 24 to 29 August in 1999, and the physiological maturity date was about 23 November in 1998 and 27 September in 1999. Visually, there was no difference in phenology between FACE and Control treatments in either season. The final sorghum grain harvest for these two growing seasons was done on 21 December in 1998 with a total 159 growth days and on 26 October 1999 with a total 133 growth days. Obviously, the sorghum crop matured significantly earlier in 1999 than 1998 because of the earlier planting and the subsequent warmer temperatures.

e. Field Management

Prior to planting in 1998, the field was laser-leveled and disked in two directions on 8 April, and the first fertilizer was applied by air at a rate of 93 kg N ha\(^{-1}\) and 41 kg P ha\(^{-1}\) on 10 June. The second application of fertilizer (only N fertilizer, UAN-32, urea ammonium nitrate) was applied on 11 September during the early growing season with irrigation at a rate of 186 kg N ha\(^{-1}\) to give a total N application of 279 kg N ha\(^{-1}\) to both FACE and Control plots for the 1998 season. After the final grain harvest on 21 December, the sorghum stubble was chopped on 12 January 1999, disked into the soil on 29 January, and disked a second time on 3 February. The field was re-leveled on 3 March. Prior to planting in 1999, the first fertilizer was applied by air on 1 June again at the same rate as 1998 (93 kg N ha\(^{-1}\) and 41 kg P ha\(^{-1}\)), and the second fertilizer was applied on 6 August with 172 kg N ha\(^{-1}\) in irrigation water to give a total of 265 kg N
ha for the 1999 season. In both years, the wet and dry treats received the same level of P and N fertilization for the seasons, and with identical P and N fertilizer levels applied to both FACE and Control plots as well.

*f. Fallow Plot*

In addition to FACE and Control plots, we reserved a 4m by 4m-fallow plot in the sorghum field for comparison. This fallow plot experienced the same plowing, fertilizing and irrigating as FACE and Control plots, but sorghum planting was excluded. We also removed any weeds growing in this plot. We assume soil respiration efflux from the fallow plot contains no root-affected decomposition of soil organic matter (SOM). Although there exists an inherent problem in taking fallow soil respiration rate as an indicator of old SOC decomposition rate in FACE or Control plots because SOM decomposition will be different with or without the belowground root system and the aboveground canopy, it is highly likely that respired CO$_2$ from fallow plots carries the precise isotopic signature of CO$_2$ derived from non-root-affected SOC degradation, or old soil humus breakdown, and also that the respiration rate represents the soil respiration without root effects.

*g. Stable C Isotopes in the System*

The pure tank CO$_2$ added to FACE plots was $^{13}$C-enriched CO$_2$ derived from a geologic deposit with $\delta^{13}$C = -4.7‰. Current atmospheric CO$_2$ has an isotopic composition with $\delta^{13}$C = -8.9‰. Air in Control plots was derived from the atmosphere with the C isotopic composition the same as atmosphere, resulting in Control C$_4$-
Fig. 4. Stable C Isotopic Composition in 1998 and 1999 Sorghum FACE Experiment. This figure showed that the air in the Control plot was derived from ambient atmosphere with \([\text{CO}_2] = 360 \ \mu\text{mol mol}^{-1} \ (\text{ppm})\) and \(\delta^{13}\text{C} = 8.93\%\), which resulted that the \(\delta^{13}\text{C}\) value of sorghum plant was about -11.9\%. The air in the FACE plot was composed of the ambient atmospheric air and pure tank \(\text{CO}_2\), resulting in FACE \([\text{CO}_2] = 560 \ \mu\text{mol mol}^{-1} \ (\text{ppm})\) and the \(\delta^{13}\text{C}\) of the FACE air = -7.56\%, and further resulting in the \(\delta^{13}\text{C}\) of the FACE sorghum plant = -10.6\% (1.3\% higher than that of the Control sorghum plant). Soil organic carbon (SOC) before the experiment was mostly derived from the frequently-cultivated \(\text{C}_3\) crops (e.g. cotton and wheat), which resulted that the \(\delta^{13}\text{C}\) values of SOC at the beginning of the experiment were -22.5\% homogeneously equal in both FACE and Control plots. The numbers in parentheses are the numbers of sample replicates. (The figure is adapted from Leavitt et al. (in revision)).
sorghum plants with δ\(^{13}\)C approaching \(-11.9\)% (Fig. 4). Air in FACE plots was composed of background air plus tank CO\(_2\), resulting in its isotopic composition being about \(-7.6\)%o. Consequently, the δ\(^{13}\)C value of sorghum in FACE plot is about \(-10.6\)%o, 1.3% less negative than that of Control sorghum. Soil organic matter in the field was mostly derived from past-cultivated C\(_3\) plants (e.g., cotton, wheat) although the last crop immediately prior to the experiment was C\(_4\) sorghum. The C\(_3\) photosynthetic pathway strongly discriminates against to \(^{13}\)CO\(_2\), whereas the C\(_4\) pathway discriminates against \(^{13}\)CO\(_2\) much less, which results in C\(_3\) plants being much more depleted in \(^{13}\)C than C\(_4\) plants. Therefore, the isotopic composition of soil organic matter at the beginning of the experiment was δ\(^{13}\)C=-22.5‰ (Fig. 4). Significant differences in the isotope composition of the C sources in the FACE experiment provided a helpful isotopic tracer for tracing C in the system and partitioning C derived from newly input sorghum residue from original SOC.

2.1.1.2. Soil Sampling

Soil samples were collected with 5.5 cm-diameter bucket augers at the beginning and end of each growth season in 1998 and 1999 experiments. The first set of samples was augered on July 20-21, 1998, after planting but prior to plant emergence. The samples included soils from depths of 0-15 cm, 15-30 cm and 30-60 cm, taken at quadrant positions 5-6 m from the center of each of the 8 rings. For each depth there were 2 between-row soil samples from each of the four replicated treatments. Each sample was place in a paper bag and subsequently air-dried. After harvest, an equivalent set of samples was collected on January 12, 1999, about 3 weeks after grain harvest. For the second growing season, we sampled soils on June 18-19, 1999, after
planting but before emergence. On Nov. 7, 1999, 12 day after grain harvest, we sampled a final complete set of soils from comparable field positions, but the soils were cored down to 100 cm and segmented at the depths of 0-15, 15-30, 30-45, 45-60, 60-80 cm. All of the soil samples, after collected from field, were transferred to the laboratory, air-dried at room temperature, and initially sieved to remove rock grains and plant fragments larger than 1 mm for carbon and isotope analyses.

2.1.1.3. Sorghum Plant Sampling

Sorghum samples were collected from the field prior to disking, when most plants had senesced. The 1998 plant samples were collected on 22 December, and the 1999 plants were collected on 27 October. The method used for collecting sorghum plant samples was same for both years: A total of 4 whole sorghum plants including seeds, leaves, stems and roots were randomly selected from each semicircular plot and dug from the ground. A total of 16 whole plants (4×4 replicates) were sampled from each treatment (FACE-wet, FACE-dry, Control-wet and Control-dry). The whole sorghum plant samples were brought to the Tree-Ring Laboratory where they were separated into roots, stems, leaves and seeds. Roots and shoots were separated from the first stem node that is just at ground level. Leaves were separated by cutting them from the stem at the leaf sheaths (leaf samples are leaf blades, and stems include leaf sheaths). Heads were removed from the first node at top stem. The separated sorghum tissues were air-dried at room temperature, ground through the 20-mesh sieve with a Millele grinder, and then oven-dried at 60 °C for 48 hours before weighing for chemical and isotopic analyses.
2.1.1.4. Soil Respiration Measurement

Soil respired CO$_2$ efflux was measured *in situ* from the soil surface at the mid-point between sorghum crop rows biweekly during August-December of 1998 and July-September of 1999, using a portable Licor-6200 CO$_2$ analyzer and PVC collars (Pendall et al., 2001). The cylindrical PVC collars, 14 cm (5.5 inch) high and 20 cm (7.9 inch) in diameter and open at both ends, were permanently inserted into the soil to a depth of ~6 cm to ensure a good seal at the PVC collar-soil interface at the beginning of growing season, where they remained undisturbed throughout the growing season. Around the middle of each PVC collar just at the ground level were three 2.6-cm-diameter holes, which allowed irrigation water and air to flow through the collars freely and maintained the same micrometeorological condition within collars as that outside. When soil respiration was measured, these three holes were carefully sealed with rubber stoppers and the top of the collars were covered with a CO$_2$ sensor plate that connected to the CO$_2$ analyzer of the Li-6200 portable system.

Measurement of soil respiration was done by recording the change in CO$_2$ concentration in the PVC collar every 4 and 10 seconds over 2-3 min. There were two duplicate PVC collars installed in each plot and two measurements were made for each collar on each sampling date. Therefore, there are 4 measurements for each plot and 8 replicate measurements for each treatment. All the measurements occurred in mornings from 09:00 to 12:00 noon local time (Mountain Standard) in the order of 1F, 1C, 2C, 2F and fallow. Respiration rate was calculated from CO$_2$ accumulation rates based on the mean value obtained from 8 repeated measurements and expressed as $\mu$mol CO$_2$ m$^{-2}$ s$^{-1}$. The first soil respiration was measured at the date when 50% of the plants had emerged.
(on August 21, 1998 and July 19, 1999) and the last measurement was conducted after sorghum entered physiological maturity (on December 9, 1998 and October 21, 1999). There were 8 measurement dates in 1998, and 7 in 1999.

2.1.1.5. Respired CO₂ Sampling

In addition to CO₂ flux measurements, we collected the respired CO₂ immediately after respiration rate was recorded, using 125-ml double-stopcock flasks that were connected in line between the CO₂ sensor plate and Li-6200 device through rubber tubing. The respired CO₂ samples were then brought to the laboratory and processed in the same way as soil air CO₂ for CO₂ concentration and isotopic analysis.

2.1.1.6. Soil Air CO₂ Sampling

Soil air CO₂ was sampled using evacuated 125-ml single-stopcock flasks at similar biweekly intervals from depths of 15 cm and 30 cm through a buried-tube array for CO₂ recovery and isotopic analysis. There were four perforated stainless steel tubes (12 cm long and 20mm-diameter) separately connected with 4-mm-diameter plastic tubing at one end and horizontally emplaced in the soil at 15 cm and 30 cm depths (two duplicate tubes at each depth) just below the sorghum crop row immediately after sorghum seeds were planted. The open end of the plastic tubing was left at the ground surface and sealed with silicone sealant between samplings to avoid atmospheric air directly exchanging with deep soil air through the tubing. When soil air CO₂ was sampled, the sealing silicone was removed temporarily and pre-evacuated 125-ml flasks were attached to the tubing with an o-ring compression connecter. Before opening the
flasks, 50-ml of air within the tube was withdrawn with a syringe to remove any atmospheric air contamination from the tube followed by 90 seconds to restore the balance of soil air in the tube and soil. After slowly opening the flask stopcocks, soil air in the tube was drawn into the flask, another 90 seconds were allowed to regain the balance of the soil air in the flask and tube, and the flask stopcock was then closed. Soil air samples were collected on 10 occasions during 1998 and 8 during the 1999 growing season.

2.1.1.7. Atmospheric CO₂ Sampling

Atmospheric air samples were collected with 2-L and 3-L evacuated flasks every 2 weeks of each growing seasons from the center of FACE and Control plots primarily in replicates 1 and 2, but occasionally in replicate 3 and 4. Sometimes, the air was drawn directly from the plot into the flasks, whereas on other occasions an integrated sample was obtained by pumping air over 10 minutes into a 10-L mylar balloon and taking the flask sample from well-mixed air within the balloon. With either the direct sample from the plot or the sample collected by balloon-reservoir method, after slowly opening the evacuated flasks to draw in the air sample, it was always allowed to stay open 90 seconds before closing the flask in order to balance the air inside and outside of flask.

2.1.2. Lab Methods

2.1.2.1. Stable Carbon Isotopes
Stable C isotopes ($^{12}$C and $^{13}$C) provide a powerful method to trace C translocation in a system. The stable C isotope composition of materials (e.g., SOC, sorghum plant tissues, gas CO$_2$ in my research) are determined by mass spectrometry, a method that separates $^{12}$C and $^{13}$C atoms on the basis of the small difference in their atomic weight. The results from a mass spectrometer are expressed in term of the permil excess $^{13}$C ($\%$), or delta $^{13}$C ($\delta^{13}$C), which was calculated relative to the international standard PDB (the international Vienna Pee Dee Belemnite) (Note: $^{13}$C is a naturally occurring stable isotope of carbon which is present in the atmosphere at concentrations of about 1% of total carbon dioxide. Isotopic composition is often expressed as a ratio between the heavier and the lighter isotope. Expression of the absolute ratio is impractical because of the large differences in natural abundance between isotopes of the same element, so it is more convenient to express an isotope ratio relative to a standard). The equation for calculation of $\delta^{13}$C is described as:

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

where, $R$ is the $^{13}$C/$^{12}$C ratio of the sample ($R_{sample}$) or the standard ($R_{standard}$) (PDB: $^{13}$C/$^{12}$C =0.0112372). For example, $\delta^{13}$C of $+10\%$ indicates that the experimental sample is enriched by 1% in atom % $^{13}$C as compared to the standard.

Two mass spectrometers used were a Finnigan-MAT Delta S dual inlet gas source isotope ratio mass spectrometer and a Finnigan Delta PlusXL continuous-flow gas-ratio mass spectrometer. The first one requires injecting pure gas CO$_2$ prepared off-line, meaning that solid samples have to be combusted and mixed gases (air) have to be purified for CO$_2$ before being run on the mass spectrometer. The second one is
equipped with an elemental analyzer (Costech), which can combust solid samples on-line, purify the byproducts and automatically inject CO$_2$ into the mass spectrometer, meaning that solid samples are directly run on this mass spectrometer, with no additional off-line pre-procedures for combustion and purification. The isotopic precision of both mass spectrometers is estimated to be 0.1-0.01‰ based on repeated measurements of several standards.

2.1.2.2. SOC Measurement

a. Removal of Inorganic Carbonates from Soil

Before measuring soil organic carbon (SOC), inorganic carbonates have to be removed. We used two methods for removing carbonates, and the results for carbon and isotopes from these two methods were comparable. Method 1 with 1N HCl: about 20-30 g of the soil sample was immersed in 150 ml of 1 N HCl in a 250-ml beaker, stirring occasionally and then sitting in the acid overnight until no CO$_2$ bubbles from the soil were produced. After the soil was acidified, a concentrated NaCl solution ($\rho \approx 1.2$ g cm$^{-3}$) was subsequently used to float persisting plant fragments, which were likewise skimmed. Soils were then rinsed with 200 ml DI water, dried at 60 °C for 48 hours 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. We applied this method to bulk soil for total organic carbon content and isotopes composition analyses.
Method—2 with strong HCl vapor: About 1-2 g of oven-dried soil sample, after being ground through a 1-mm sieve and examined under 20 × magnification to remove recognizable fine plant fragments, was placed in 5-ml open glass vials. The vials were placed in the wells of a microtiter plate, sufficient water was added to each vial to moisten the soil to approximately field capacity, and the microtiter plate was then placed in a vacuum desiccator. A beaker (150 ml) with 100 ml of concentrated (12 M) HCl was also placed inside the desiccator. The samples were exposed to strong HCl vapor for 12 hours. After being acidified, the samples were removed from the desiccator and dried at 60 °C for 48 hours. This method was based on Harris et al. (2001). This new method, compared to Method—1, is simple and fast, and can avoid loss of acid-soluble organic C. We applied this method to partitioned soil samples for labile and recalcitrant C content and isotope composition analyses.

b. Combustion of Soil

Bulk soil samples collected from FACE and Control plots were analyzed for C content and isotopic composition before 2001, run on the Finnigan-MAT Delta S mass spectrometer that requires combusting of solid samples off line. About 100-200 mg of soil sample free of carbonates were placed in a quartz tube (0.9 cm in diameter × 23.5 cm in length), about 250 mg of CuO powder (1 cm high in the tube) that was preheated at 900 °C for at least 2 hours was mixed with the soil, and then a narrow glass plug (0.3 cm in diameter and 5 cm in length) and a 1-cm length of Ag foil were inserted into the larger tube above the soil. The tube was plugged with glass wool, and then placed on a vacuum line for at least 12 hours. After evacuating, the tube with soil sample was
sealed and cut from the line just under the glass wool, and was combusted in oven at 900 °C for 2 hours. It was then switched to 650 °C for another 2 hours. This method followed that of Boutton (1991) and Leavitt et al. (in revision).

c. \textit{CO}_2 \textit{Purification}

The gas from soil combustion contains several gases from which pure CO\textsubscript{2} needs to be isolated before being run on mass spectrometer. To purify CO\textsubscript{2}, the mixed gas was first admitted sequentially into one cold (-80 °C) ethanol trap and two liquid N\textsubscript{2} traps (-196 °C) on a vacuum line. The gas was allowed to stand in traps for 5 minutes to ensure that all water was trapped in the ethanol trap and CO\textsubscript{2} was trapped within the liquid N\textsubscript{2} traps. Then, the vacuum line at the end of one side of the liquid N\textsubscript{2} traps was opened. The gas stream was slowly bled through the traps until all non-freezing gases were completely removed and the line was evacuated. CO\textsubscript{2} trapped in the two liquid N\textsubscript{2} traps was vaporized by heating, after closing the vacuum line, and was re-trapped from the line into 10-ml vials with liquid N\textsubscript{2}. This procedure separates pure CO\textsubscript{2} from H\textsubscript{2}O and other gases in the sample without fractionating the carbon isotopes.

d. \textit{Carbon Content Measurement}

The yield of the purified CO\textsubscript{2} was measured with a manometer on a vacuum line before running the sample on a Finnigan Delta-S mass spectrometer. The principle of measuring CO\textsubscript{2} yield is according to the Ideal Gas Law: \( PV = nRT \) or \( n = PV / RT \). When temperature \( T \) in Kelvin, pressure \( P \) and volume \( V \) are known after the purified CO\textsubscript{2} is trapped in the vacuum line, the number of moles of CO\textsubscript{2} and therefore
carbon can be calculated based on the equation. \( R \) is a universal gas constant. In SI units (Système International), where pressure is expressed in pascals and volume in cubic meter, \( R = 8.31 \text{ J mol}^{-1} \cdot \text{K} \). If the pressure is expressed in atmosphere and the volume is given in liter \( (1 \text{L} = 10^3 \text{ cm}^3 = 10^{-3} \text{ m}^3) \), then \( R = 0.0821 \text{ L} \cdot \text{ atm} \cdot \text{mol}^{-1} \cdot \text{K}^{-1} \).

e. Isotopic Analysis

After yield measurement, the isotopic composition of the pure CO\(_2\) was analyzed on a Finnigan-MAT Delta S dual inlet gas source isotope ratio mass spectrometer. The mass spectrometer gives a result directly in \( \delta^{13}C \). The \( \delta^{13}C \) values of bulk SOC were measured on this mass spectrometer, after off-line sample combustion and CO\(_2\) purification. The \( \delta^{13}C \) values of incubated SOC and labile and recalcitrant C were measured on a new Finnigan Delta PlusXL continuous-flow gas-ratio mass spectrometer by directly dropping about 30-50 mg of the soil sample loaded into small Sn (tin) capsules into the elemental analyzer. This new mass spectrometer produces results for both total C content (%) and \( \delta^{13}C \) value, which were equivalent to those of organic carbon because we had pretreated the soil to remove carbonates.

2.1.2.3. Respired CO\(_2\) and Soil Air CO\(_2\) Content and Isotopic Analysis

The respired CO\(_2\) and soil air samples collected from FACE and Control plots were processed to purify CO\(_2\) immediately after they were brought in laboratory in 1998 and 1999. The methods used for CO\(_2\) purification and yield measurement were exactly the same as those for the gas from the combustion of soil samples. The mass
The spectrometer used for isotopic analysis was the Finnigan-MAT Delta S dual inlet gas source isotope ratio mass spectrometer.

2.1.2.4. Sorghum Plant Tissues C Content and Isotopic Analysis

Whole sorghum plant materials (about 4 mg of each sample) were combusted in a recirculating microcombustion system in the presence of excess O$_2$, and the CO$_2$ was isolated cryogenically just like with the CO$_2$ combusted from soil samples. The purified CO$_2$ was analyzed on a Finnigan-MAT Delta S dual inlet gas source isotope ratio mass spectrometer for isotopic composition. Sorghum tissues (leaf, stem and root), after being ground through a 20-mesh sieve and oven-dried at 60 °C for 48 hours, were analyzed on the Finnigan Delta PlusXL continuous-flow gas-ratio mass spectrometer by injecting about 0.6-0.8 mg of the tissues in small Sn (tin) capsules, for C content (%) and isotopic composition ($\delta^{13}$C).

2.1.2.5. Sorghum Biochemical Composition Analyses

a. Phenolic Acids

Phenolic acids of sorghum residue materials (50 mg) were extracted with 5 ml of 1 M NaOH for 16 h on a reciprocal shaker at ambient temperatures as described in detail by Martens (2002). Following extraction, the mixture was centrifuged (2300 rpm, 20 min), and rinsed with 1 ml water. The supernatants were carefully poured into a disposable glass test tube and heated at 90 °C for 2 h to release the conjugated phenolic acids. After the heated extract was cooled, the samples were titrated with 4 M methanesulfonic acid to pH < 2.0, diluted to 10 ml with DI water, and centrifuged to
remove the precipitate. An aliquot (2-3 ml) of the supernatants was passed through a conditioned Varian (Varian Assoc., Harbor City, CA) Bon Elut PPL solid-phase exztraction tube (The PPL tubes were conditioned by first passing 2 ml of ethyl acetate followed by 2 ml water) at ~5 ml min$^{-1}$ attached to a Visprep (Supelco, Bellefonte, PA). The tubes were then placed under a vacuum (~60 kPa) until the resin was thoroughly dried after which the phenolic acids (PAs) were eluted with 1 ml of ethyl acetate into gas chromatography autosampler vials for quantification by a Hewlett-Packaed 1800A GCD gas chromatograph equipped with a HP-Ultra 1 capillary column (25-m length, 0.2-mm column i.d., 0.33-um film thickness) and a mass selective (MS) detector.

\[ b. \textit{Amino Acids} \]

Amino Acids were determined by digestion of the materials (20 mg) in screw-top test tubes with 2 ml of 4 M methanesulfonic acid, and the mixtures were autoclaved for 16 h at 121 °C (104 kPa). Following digestion, the samples were titrated to pH 4-5 with 5 M KOH and centrifuged to remove precipitate, and then an aliquot of supernants was diluted for analysis of Amino acids and amino sugars on a Dionex DX-500 (Dionex Corp., Sunnyvale, CA) ion chromatograph equipped with a 25 µl injection loop and an AminoPac PA10 guard and analytical column (250 mm x 2 mm i.d.). Pulsed amperometric detection was by a Dionex ED-40 electrochemical detector set in the integrated pulsed mode with a disposable gold working electrode. The amino acid standards [AA kit (catalog no. LAA-10) and standard solutions (catalog no. AA-S-18)] were obtained from Sigma Chemical Co., St. Louis, MO, and were used to map retention times and detector response.
c. Uronic Acids

Uronic acid content was determined enzymatically by the method described in Martens and Frankenberger (1990). To solubilize uronic acids, sorghum tissue samples (200 mg) were treated with 0.3 ml of 6 M H$_2$SO$_4$ to which was added 6.9 ml of DI (deionized) water to produce 7.2 ml of 0.25 M H$_2$SO$_4$. The mixtures were then autoclaved for 30 min at 121 °C, and after cooling centrifuged to remove precipitate (rinsed with 1 ml DI water and centrifuged again). The supernatants were combined into screw-top test tubes, titrated to pH 4-5 with NaOH and then diluted to 10 ml with DI water. A 1-ml aliquot was diluted to 5 ml with DI water, treated with the enzymes pectolyase (3 units ml$^{-1}$) and B-D-glucuronidase (30 units ml$^{-1}$) at pH 6.8 (phosphate buffer) and then the mixtures were incubated overnight at ambient temperatures (16 h). The enzyme-extract mixtures were passed through an activated strong anion (3-quanternary propylammonium, Cl$^{-1}$) exchange column and rinsed with 3ml water. The uronic acids were eluted with several milliliters of 0.1 M NaCl (pH 8.0) and quantified by ion chromatography with pulsed amperometric detection.

d. Hemicelluloses

Hemicellulose carbohydrates were extracted at room temperature from 20 mg samples of sorghum residue materials in screw-top test tubes (15x125 mm) with 0.8 ml of 6 M H$_2$SO$_4$, shaken with mild vortex mixing for 30 min, diluted with 4.2 ml of DI water to 1 M H$_2$SO$_4$, and then autoclaved at 121 °C (104kPa) for 30 min. After digestion and cooling, the samples were centrifuged, washed with two aliquots of 1 ml DI water and centrifuged again between each rinse. The three supernatants were
combined in 14 ml Falcon Sterile Tubes, titrated to pH 5.5-6.5 with 5 N NaOH and then diluted to 10 ml with DI water for the analysis of separated hemicellulose carbohydrate on a DX-500 ion chromatograph equipped with a CarboPac PA10 column.

\[ e. \] \textit{Cellulose}

Cellulose was analyzed using the residues after hemicellulose extraction. The wet residues were oven-dried at 60 °C overnight, 300 µl of 18 M H\textsubscript{2}SO\textsubscript{4} was added to each sample, shaken and allowed to wet thoroughly for 30 min. After cellulose solublization in this strong acid, the samples were diluted to 1.5 M H\textsubscript{2}SO\textsubscript{4} with an additional 3.6 ml of DI water, autoclaved at 121 °C (104kPa) for 30 min, and then centrifuged (2100 rpm, 20 min) to remove precipitation (rinsing once with 1 ml of DI water and centrifuging again). The supernatants were combined into 14 ml Falcon Sterile tubes, neutralized to pH 5.5-6.5 with 5 N NaOH, and then diluted to 10 ml with DI water. Monosaccharides released from sample cellulose solublization were identified and quantified on a Dionex DX-500 ion chromatograph.

\[ f. \] \textit{Lignin}

For the lignin assays, residue samples (300 mg) were treated with 15 ml of 72% sulfuric acid at 20 °C for 2 hours, with frequent stirring. The mixture was then transferred to a 2000-ml flask and 560ml of water was added to decrease the acid concentration to 3% (w v\textsuperscript{-1}). The hydrolysate was boiled for 4 hours, maintaining constant volume by covering the flask with a 50-ml flask for condensation and by frequently adding hot water as well. After boiling 4 hrs, the solution was allowed to
cool down, and the insoluble materials (lignin) settled. The lignin was filtered with a 30-ml filtering crucible mounted on a 2000-ml filtering flask equipped with a vacuum line, and then washed free of acid with hot water. The crucible with lignin was dried in an oven at 105±3 °C to constant weight and then the lignin was collected. The results were expressed as mg g⁻¹ of a total dry mass.

2.1.2.6. Residue Incubation

To evaluate how the biochemical composition affects the decomposition rate of the sorghum tissues, we conducted a 79-day laboratory incubation of the soil amended with leaves, stems and roots of sorghum collected from FACE and Control treatments.

a. Incubation Procedures

Twenty grams of homogenized soil that was collected from FACE experiment, air-dried at room temperature and then sieved through 1 mm sieve, were thoroughly mixed with 50 mg of one type of sorghum tissues (leaf, stem and root), which is equal to the addition of 6.5 tons (1 ton = 1000 kg) fresh organic materials to one hectare soil (assuming a bulk density of 1.3 g cm⁻³ and a depth of 20 cm; 1 hectare = 10,000 m² = 10⁸ cm²; 1.3 g cm⁻³ × 20 cm × 10⁸ cm² = 2.6 × 10⁹ g soil = 2.6 × 10⁶ kg soil). The mixtures of soil and sorghum tissues were placed into 250 ml flasks, which were then tightly covered with rubber stoppers through which two tubing outlets were made, one for respired CO₂ air going out and the other for CO₂-free fresh air going in, which were kept closed most of the time with clips. Prior to the experiment, the volume of each
flask excluding the stopper was exactly measured by filling with a known volume of deionized (DI) water.

Duplicate flasks were set up for all sorghum leaf, stem and root additions, together with 2 control flasks containing soil only. The soil within each flask was kept at 95% of its water holding capacity through the experiment (79 days) by weekly addition of DI water to a pre-determined weight. The incubation was started by evenly adding 4 ml (pre-determined based on moisture of 20 g soil) of DI water to each flask, flushing the unit with CO$_2$-free air and placing them on a table at the 23-25 °C of room temperature.

b. Decomposition Rate Measurement

To determine soil organic matter decomposition rate, an infrared gas analysis meter connected to a computer system was employed to record the microbially respired CO$_2$ concentration as respired CO$_2$ in the flask was flushed through the meter from the tubing outlet with CO$_2$-free air from the other tubing outlet. Flasks were thus aerated at the end of each measurement. The first measurement was conducted after 6 hours of incubation (near the end of microorganism lag growth), and then measurements were conducted at 2-hour intervals during the first week. Thereafter measurements were reduced to once per day and once per week during the 79-day incubation. Immediately after each measurement, the two tubing outlets in the stopper of the flask were closed again with clips and the last CO$_2$ concentration within the flask was recorded, from which the next measured [CO$_2$] was subtracted for the calculation of net CO$_2$ produced during each incubation period since the last measurement. Rates of respired CO$_2$
production were calculated as the cumulative amount of CO₂-C with time. Net CO₂-C production attributable to the decomposition of new added organic matter at each measuring time was calculated by subtracting the mean value of the respired [CO₂] of 2 controls (soil alone) from each measurement of the sample (soil + tissues), with cumulative respired CO₂-C being calculated according to: [CO₂] × flask volume ÷ 24.34 × 12. At the end of the incubation experiment, the incubated soils were analyzed for organic carbon content and stable isotopic composition.

2.2.2.7. 6 N HCl Hydrolysis

Acid hydrolysis with 6N HCl was used to partition SOC into labile and recalcitrant C pools. The hydrolysis 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 Campbell et al. (1967) and Leavitt et al.(1996). Ten g of soil were placed into a 500 ml round-bottom flask with 150 ml of 6N HCl. With a water-cooled condenser installed above the flask, the mixture was heated to boiling with an electric heating mantle for ca. 18 hours. After hydrolysis, the mixture was allowed to cool down and centrifuged at 2500 rpm for 15-20 minutes. Supernatant liquid was decanted to a pre-weighed 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 pre-weighed 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, and analyzed for carbon and $\delta^{13}C$.

2.1.3. Mathematic Methods

2.1.3.1. Isotopic Partitioning

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:

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

where, $\delta^{13}C_{\text{sample}}$ and $\delta^{13}C_{old}$ are the isotopic signatures of SOC after and before the sorghum FACE experiment, respectively. $\delta^{13}C_{new}$ 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, although Mary et al. (1992) reported that a small isotopic effect occurs in decomposition processes. The fraction of original (old) SOC was calculated as: $f_{old} = 1 - f_{new}$. Here, we assumed all SOC after two-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:

$$C_{new\%} = f_{new} \times C_{total}, \text{ and}$$

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

When partitioning root respiration from soil air CO$_2$, the natural difference of the $\delta^{13}C$ values in soil air CO$_2$ and its source was considered. Soil CO$_2$ $\delta^{13}C$ values are
naturally more enriched in $^{13}$C than the organic matter from which they are derived. This fractionation is caused by the difference in the diffusivities of the two isotopic species (the lesser diffusivity of $^{13}$CO$_2$ relative to $^{12}$CO$_2$). At steady-state conditions, the diffusive fractionation is eliminated by the imposition of mass balance and the $\delta^{13}$C value of soil gas reservoir approaches a value $\sim$ 4.4‰ higher than the production (Amundson et al., 1998; Cerling, 1984). Therefore, prior to using the isotopic mixing model to partition soil air CO$_2$, the $\delta^{13}$C value of soil air samples were corrected by subtracting 4.4‰ for this kinetic fractionation produced during diffusion.

2.1.3.2. Mean Residence Time

To determine the decomposition rate ($k$) and mean residence time (MRT) of SOC, the following single exponential decay model was used in this present study:

$$X_t = X_o e^{-kt}, \quad \text{and}$$

$$MRT(\text{yr}) = \frac{1}{k} = \frac{t(\text{yr})}{\ln(X_t/X_o)}$$

where, $X_t$ is the original SOC remaining after t years; $X_o$ is the SOC content prior to the CO$_2$ experiment; $k$ is the decomposition constant; $t$ is the time elapsed since CO$_2$ fumigation. 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.
2.1.3.3. Statistics Analysis

The significance of CO$_2$ and water effects on resulting parameters was tested by analyses of variance (ANOVA) and paired-t test. A one-way ANOVA was used to test the difference of CO$_2$ or water effect on soil respiration, soil air CO$_2$ concentration, and decomposition rates from laboratory incubation. A two-way ANOVA was further used to test the main effect of CO$_2$ interacting with water on these parameters. A paired t-test was applied to test the separate CO$_2$ and water effects on sorghum plant chemical composition, total SOC, bulk soil MRT, labile and recalcitrant C, and C$_{r}$/C$_{total}$ and C$_{l}$/C$_{total}$ ratios. To evaluate correlation between decomposition rate and chemical composition, regressions were applied to cumulative CO$_2$-C and new input C against the tissue biochemical parameters, and these regressions were tested across all tissues with n=12 (3 tissues $\times$ 2 CO$_2$ levels $\times$ 2 duplications). In assessing the relationships between MRT of bulk SOC and ratios of C$_{r}$ and C$_{l}$ to C$_{total}$, 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. Additionally, one-way ANOVA was also performed to determine the significant differences of degradation between tissues of leaf, stem and root, and the differences of old and new soil organic C left in the incubated soil between treatments. All error estimates presented in tables and error bars in figures are one standard deviation. Statistically significant differences were considered at the $\alpha = 0.05$ probability level. All statistical procedures were conducted using Minitab statistical software (Version 13, Silicon Graphics).
2.2. Summary and Concluding Remarks

2.2.1. Seasonal Soil Respiration and Soil Air CO₂ under Elevated Atmospheric CO₂ in a C₄-Sorghum (Sorghum bicolor (L.) Möench) Agroecosystem.

The primary goal of this first study was to investigate the variations of soil respiration in response to elevated CO₂. This was done by regular field measurement of soil respiration with a Licor-6200 and regular sampling of soil CO₂ air at depths of 15 and 30 cm through permanently installed tubing. Two-year average soil CO₂ efflux from elevated plots was 3.3 \( \mu \text{mol m}^{-2} \text{s}^{-1} \), about 13% greater than that from ambient plots (accept Hypothesis 1), but the seasonal pattern in 1998 was different from 1999. Soil CO₂ efflux under elevated CO₂ in 1998 increased from 3% to 107% significantly throughout the growing season, whereas in 1999 large increases in soil CO₂ efflux occurred during later stages of growth, whereas before sorghum heading the CO₂-enriched plot had lower soil CO₂ efflux than the control. To further understand the belowground C cycling, carbon isotopic (δ¹³C) analysis of soil CO₂ air collected from the soil profile was used to resolve root respiration and old SOC decomposition in the respired soil CO₂. The results indicate that although elevated CO₂ increased soil CO₂ efflux significantly, about 55% of the increased CO₂ flux was derived from root respiration and 45% came from old SOC decomposition. Compared with control over two growing seasons, elevated CO₂ increased root respiration by an average of 36%, but the effect on old SOC decomposition was not significant, suggesting that increased plant root respiration did not stimulate original soil organic matter mineralization (reject Hypothesis 2).
2.2.2. Biochemical Compositions of Sorghum (*Sorghum bicolor* (L.) Möench) Tissues in Response to Elevated Atmospheric CO$_2$ with Adequate and Deficient Water Supply.

The primary goal of this second study was to determine the major biochemistry of senesced leaves, stems, and roots of sorghum grown under elevated CO$_2$, and the interactive effects of CO$_2$ and water on sorghum chemical composition. I focused on the chemical compounds of amino acids (AAs), uronic acids (UAs), hemicellulose carbohydrates (HCCs), cellulose carbohydrates (CCs), lignin, non-lignin phenolics (NLPs), carbon (C), nitrogen (N) and C:N ratio. This is because they are ubiquitous in terrestrial higher plants, because they have exhibited sensitivity to elevated CO$_2$, and also because they are critical to plant residue decomposition in the soil ecosystems, and thus will influence the fate of atmospheric C sequestered by terrestrial ecosystems. These analyses were done by using high-performance anion-exchange chromatography (HPLC) with pulsed amperometric detection analysis (HPAEC-PAD) performed on a DIONEX DX-500 (*Dionex Xorp. Sunnyvale, CA*). The results showed that elevated CO$_2$ and water-stress treatments elicited changes in sorghum residue biochemistry, reducing AAs and HCCs by 7% and 8%, respectively, and increasing CCs and lignin by 49% and 5%, respectively (support Hypothesis 4). NLPs were significantly higher in FACE roots, but no significant difference emerged between FACE leaves and stems and their control counterparts (support Hypothesis 4). Soil water stress largely enhanced the CO$_2$ effect on sorghum biochemical composition. For UAs, elevated-CO$_2$ effects were modified by soil moisture conditions with a 7% reduction of UAs in FACE-wet and up to 6% increase in FACE-dry sorghum residues. The C:N ratio of
sorghum tissues was not affected by CO$_2$, but was substantially lower under water stress. The results indicated CO$_2$-elicited changes in sorghum tissue chemistry, which will carry over to sorghum residue/litter as an input of new soil organic matter, play a pivotal role in affecting the dynamics of sorghum residue/litter decomposition.

2.2.3. Tissue Chemistry of Sorghum Grown under Elevated Atmospheric CO$_2$

Impacts SOC Decomposition Rate.

The primary goal of this third study was to evaluate how the biochemical composition change because of CO$_2$ affects the decomposition of sorghum residues. This was done by laboratory incubation of soil amended with leaves, stems, and roots of sorghum tissues derived from elevated and ambient CO$_2$ treatments. The results showed that the cumulatively respired CO$_2$-C from the soil amended with FACE sorghum tissues was on average 7.3% significantly less than that from the soil amended with control sorghum tissues, especially for FACE-root-amended soil that respired CO$_2$-C was 12.7% lower than that released from control-root amended soil over all incubation days, suggesting soil microbial activity was not enhanced by the addition of FACE sorghum tissues as large as by Control tissues, and further indicating CO$_2$-induced alterations in C quality reduced the decomposition rate of the residues (support Hypothesis 3 and accept Hypothesis 5). Carbon isotopic ($\delta^{13}$C) analysis revealed that on average 9.1 mg and 7.2 mg of new carbon were incorporated into the 20g of the soil amended with FACE and Control tissues, respectively, with 26.4% more new C input to the soil with added FACE tissues compared to with Control tissues after 79-d incubation, indicating that FACE sorghum tissues degraded slower than Control tissues.
(support Hypothesis 5). The correlation between soil respired CO$_2$-C with sorghum tissue composition indicated that non-lignin phenolics ($r^2 = 0.93, p = 0.002$), and lignin ($r^2 = 0.9, p = 0.004$) are the most important factors to reduce sorghum tissue decomposition, and C:N ratio was not found to be significantly related to decomposition (accept Hypothesis 5 and Hypothesis 3).

2.2.4. Dynamics of Labile and Recalcitrant Soil Carbon Pools in a Sorghum Free-Air CO$_2$ Enrichment (FACE) Agroecosystem

The primary goal of this last study was to consider how elevated CO$_2$ impacts soil C sub-pools and the mean residence time (MRT) of bulk SOC. The 6 N HCl hydrolysis method was used to partition soil C pool into labile and recalcitrant pools, and a single exponential decay model was used to calculate the MRT of bulk SOC. The results showed that on average 53% of the final soil organic C in the FACE plot over two year experiments was in the recalcitrant C pool and 47% in the labile pool, whereas in the Control plot 46% and 54% of C were in recalcitrant and labile pools, respectively, indicating that elevated CO$_2$ encouraged more SOC to transfer into the slow-decay C pool. Isotopic mixing models reveal that increased new sorghum residue input to the recalcitrant pool mainly account for the increased size of bulk soil recalcitrant C pool, especially for the upper soil horizon (0-30 cm) where new C in FACE wet and dry soil recalcitrant pools was 1.7 and 2.8 times as large as that in Control wet and dry recalcitrant pools, respectively. The MRT of bulk SOC at the depth of 0-30 cm increased from 14-22 yrs in the Control plots to 15-58 yrs in FACE plots, which 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, further confirming that elevated CO₂ reduced the decomposition rate of SOC (support Hypothesis 6). The reduced SOC decomposition might be related to more recalcitrant new sorghum residue input and reduced old SOC decomposition occurrence under elevated CO₂ compared to ambient CO₂.

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APPENDIX A

Seasonal Soil Respiration and Soil Air CO₂ under Elevated Atmospheric CO₂ in a C₄-
Sorghum (Sorghum Bicolor (L.) Möench) Agroecosystem

L. Cheng, S.W. Leavitt, T. J. Brooks, A.D. Matthias, D.G. Williams, T. L. Thompson, B.
A. Kimball and P. J. Pinter
Abstract

Soil respiration directly transfers soil organic carbon (SOC) to the atmosphere and may determine the success of soil carbon sequestration schemes. However, the effects of elevated atmospheric CO$_2$ on soil respiration are still poorly understood, especially in the open-field agro-ecosystems. Our study investigated the variations of soil respiration under elevated CO$_2$ with well-watered treatment during 1998 and 1999 growing seasons at the sorghum Free-Air CO$_2$ Enrichment (FACE) experiment at the University of Arizona Maricopa Experimental Farm. Soil respiration was measured at the soil surface with a Licor-6200 and soil air was sampled at depths of 15 cm and 30cm at biweekly intervals. Two-year average soil CO$_2$ efflux from elevated CO$_2$ (ambient + 200 µmol mol$^{-1}$) plots was 3.3 µmol m$^{-2}$s$^{-1}$, about 13% greater than that from control (ambient CO$_2$ concentration ≈360 µmol mol$^{-1}$) plots, but the seasonal pattern in 1998 was different from 1999. Soil CO$_2$ efflux under elevated CO$_2$ in 1998 increased from 3% to 107% throughout the growing season. In 1999 large increases in soil CO$_2$ efflux occurred during later stages of growth, whereas before sorghum heading the CO$_2$-enriched plot had lower soil CO$_2$ efflux than the control. To further understand the belowground C cycling, carbon isotopic ($\delta^{13}C$) analysis of soil air collected from the soil profile was used to resolve root respiration and old SOC decomposition in the soil CO$_2$ efflux. Our results indicate that although elevated CO$_2$ increased soil CO$_2$ efflux significantly, about 55% of the increased CO$_2$ flux was derived from root respiration and 45% came from old SOC decomposition. Compared with control over two growing seasons, elevated CO$_2$ increased root respiration by an average of 36%, but the effect on old SOC
decomposition was not constant---stimulating SOC decomposition by 28% in 1998 and reducing it by 3% in 1999. Overall, our results do not support the “priming effect” hypothesis, but do support the stimulation of root respiration by elevated CO₂.

Introduction

Soil respiration is one of the primary pathways through which terrestrial ecosystems exchange carbon with the atmosphere, and understanding its response to elevated atmospheric CO₂ is essential to forecast carbon balance in terrestrial ecosystems in the future CO₂-enriched environment. Many studies have indicated that photosynthetic carbon uptake by plants is generally increased under elevated CO₂ with estimated 30-40% potential increases in net primary productivity (NPP) of agro-ecosystems when atmospheric [CO₂] is doubled (Berntson, 1996; Kimball et al., 2002; Poorter, 1993). This raises the possibility that terrestrial ecosystems will sequester more carbon (C) as atmospheric CO₂ continues to increase. However, long-term C sequestration depends on incorporation of photosynthates into ecosystem pools with long mean residence time and eventually depends on C inventory in the soil because a large proportion of CO₂ fixed in terrestrial ecosystems is eventually allocated belowground, to roots (Rogers et al., 1994b) and soil (Jones et al., 1998). The most important aspect of C sequestration in soil is the response of belowground processes, such as soil respiration, rhizosphere respiration and soil organic matter (SOM) decomposition, to elevated CO₂ concentrations. Currently, the prevalent hypothesis is the “priming effect” that states that under elevated CO₂ SOM decomposition will be stimulated by the addition of labile organic carbon derived from CO₂-enhanced plant growth, potentially leading to increased soil respiration and thus soil
C loss to atmosphere (Delenberg and Jager, 1989; Luo et al., 1996). Unfortunately, this hypothesis is based on the results of relatively short-term studies of aboveground responses to elevated \([\text{CO}_2]\) so its application to long-term storage of carbon in the soil is uncertain. In order to more precisely predict C sequestration of terrestrial ecosystems under elevated \([\text{CO}_2]\), belowground processes must be explored.

Actually, the \(\text{CO}_2\) effects on belowground processes are poorly known, and the studies of the responses of soil respiration to elevated \([\text{CO}_2]\) are few in number and generally based on pot or chamber experiments. Results from field studies have shown that soil respiration response to elevated \([\text{CO}_2]\) varies among ecosystems. Significant increase in soil respiration (50%) was detected in a mid-successional lowland forest soil exposed to 693 μmol mol\(^{-1}\) \([\text{CO}_2]\) compared to 352μmol mol\(^{-1}\) \([\text{CO}_2]\) (Ball et al., 2000). Seasonal average respiration of sandstone grassland at 500 μmol mol\(^{-1}\)\(\text{CO}_2\) was increased by 42% compared to the ambient \(\text{CO}_2\) treatment (Luo et al., 1996). In our prior free-air \(\text{CO}_2\) enrichment (FACE) experiment with cotton and wheat at the Maricopa Agricultural Center, Arizona, \(\text{CO}_2\) enrichment significantly increased soil respiration (Nakayama et al., 1994; Pendall et al., 2001). However, soil respiration rate in the Alaskan tundra was not significantly affected by either long- or short-term \(\text{CO}_2\) enrichment (Oberbauer et al., 1986). In a \textit{Populus} forest, soil respiration was not affected by doubling atmospheric \(\text{CO}_2\) (Randlett et al., 1996). Variations in soil respiration rates under elevated \(\text{CO}_2\) may be associated with other ecosystem attributes and processes.

It should be noted that the great majority of recent studies on the effects of elevated \(\text{CO}_2\) on terrestrial ecosystems were carried out on \textit{C}_3 plants. Few studies on belowground responses of \textit{C}_4 plants to elevated \(\text{CO}_2\) have appeared in the literature. \textit{C}_4
plants represent about half of the world’s grass species and contribute ~20% of global grass primary productivity (Lloyd and Farquhar, 1994). Uncertainty regarding their response to rising atmospheric [CO₂] may limit predictions of future global change impacts on C₄-dominated ecosystems. These uncertainties have not yet been considered rigorously because of expected low C₄ responsiveness to elevated CO₂ based on photosynthetic theory and early experiments. Recent studies make it increasingly clear that response of C₄ species to elevated CO₂ is not as clearcut as previously thought, and that many C₄ plants show significant photosynthetic and growth responses to CO₂. Wand, et al. (1999) reported that both C₄ and C₃ species increased total biomass significantly in elevated CO₂ by 33% and 44%, respectively. Poorter (1993) found an average growth enhancement of 22% for C₄ species. Owensby et al. (1993) have also reported significant aboveground biomass increases in the C₄ component of a tall grass prairie site exposed to elevated CO₂. At the leaf level, significant stomatal closure, increased leaf water-use efficiency and higher carbon assimilation rates were confirmed in both C₃ and C₄ species (Wand et al., 1999). Questions remaining in these studies are about belowground response to elevated CO₂ in C₄ ecosystems, especially CO₂ effects on root respiration and SOC decomposition, which determine C fate in the soil system. This is one of the least well-understood but most important areas of study on enhanced-CO₂ effect on terrestrial ecosystems.

Soil respiration represents the flux of biologically generated CO₂ from the soil surface into the overlying atmosphere (Buyanovsky and Wagner, 1995; Cerling, 1991). The sources of CO₂ in the soil respiration include live root respiration, litter (e.g. exudates, sloughed-off material, dead roots and other dead plant tissues) decomposition
and soil humus breakdown (Buyanovsky and Wagner, 1995). The respired CO₂ coming
from these different sources will determine the C storage potential in the soil. Generally,
more CO₂ derived from root-related activity (e.g. root metabolism and heterotrophic
decomposition of exudates, sloughed-off material, and dead roots) indicates higher plant
growth and more organic C input to the soil, whereas more CO₂ coming from soil organic
carbon (SOC) breakdown will result in more SOC loss and the final reduction of soil C
storage (Lambers, 1996). Obviously, it is equally critical for estimating feedback between
global change and soil processes to study the influence of CO₂ enhancement on total soil
respiration and its contribution of the sources as well.

Compared to the number of papers on bulk soil respiration response to elevated
CO₂, very little research has been conducted on partitioning of this respiration. The main
obstacle is that with current routine methods, it has proven quite complex to determine
the relative contribution of various sources to total soil CO₂ fluxes measured in the field,
especially the CO₂ contribution from root respiration and decomposition of newly-formed
plant matter, which are distinguished with more difficulty (Pendall et al., 2001). Actually,
all the studies, no matter what techniques they used, include part of microbial respiration
of newly-formed materials from live roots in root respiration, as reflected by the wide
range of published estimates of root respiration and SOC decomposition potentials for
various ecosystems from –2% (Nobel and Palta, 1989) to 74% (Lekkerkerk et al., 1990).
Much of the variability in these estimates might also be generated by the variety of
measurement techniques used. Current techniques for separating root respiration and
SOC decomposition from total respiration include root exclusion (Hendrikson and
Robinson, 1984), plot trenching (Bowden et al., 1993; Ewel et al., 1987), clear cutting
(Nakane et al., 1996), hydroponics (Atkin et al., 1996; Poorter et al., 1995) and isotopic methods (Lin et al., 2001), and they are all involved in soil disturbance and an extrapolation of the rates either from individual roots to the whole system, or from lab/greenhouse to natural environments. Soil respiration and its source distribution may be modified when the environment is disturbed because of concomitant change in environmental CO₂, O₂ and interaction between root activity and SOC decomposition (Berntson and Bazzaz, 1996a). Ideally, estimates of soil respiration and its sources are made with minimal alteration of natural environments.

In our study, we compare soil respiration in a fallow plot with no roots with root-affected respiration in C₄-sorghum FACE and Control plots in an open-field agro-ecosystem to partition the total soil-respired CO₂ into two C pools: root respiration and SOC decomposition, based on specific C isotope imprint carried in these C sources. Here, we define root respiration as the sum of live root respiration and microbial respiration of newly-formed labile C such as root exudates, sloughed-off material and dead roots that were derived from live roots during the current growing season, known as ‘rhizosphere respiration’. SOC decomposition in this paper includes pre-existing litter decomposition and old pre-existing soil humus decomposition. The objectives of this study were (1) to quantify the effect of elevated CO₂ on soil respiration in a C₄-sorghum agro-ecosystem, (2) to estimate the relative contributions of root (i.e. rhizosphere) respiration and SOC decomposition to total soil respiration, and (3) to elucidate the mechanisms of soil belowground processes involved in this partition, which are critical for understanding terrestrial C sequestration under the elevated CO₂ condition.
Materials and Methods

Field description

This research was conducted in 1998 and 1999 by using an open-field CO₂-applied ring system at the C₄-sorghum Free-air CO₂ Enrichment (FACE) experiment at the University of Arizona Maricopa Experimental Farm (AZ, USA) at which the field soil was classified homogenously as Trix clay loam soil (fine-loamy, mixed calcareous hyperthermic typic torrifluvents) (Post et al., 1988). The detailed methodology of this FACE experiment can be found in Kimball et al. (1999), Ottman et al. (2001) and Wall et al. (2001). Briefly, the experiment was designed with two CO₂ treatments--- high CO₂ (ambient+200 μmol.mol⁻¹) as “FACE”, low CO₂ (ambient 360 μmol.mol⁻¹) as “Control”, and four replicates. Hence, there was a total 8 of 25-m-diameter rings, with four FACE and four Controls. These circular plots were constructed from PVC pipes that were placed in the field shortly after sorghum planting. Additional pure tank CO₂ was applied in FACE plots continuously from emergence to plant maturity to raise CO₂ concentration in FACE plots to ~560 μmol.mol⁻¹. Identical air blower systems were also installed in the non-CO₂-enriched ambient Control plots to reduce the micrometerological error that could be caused by reduced air circulation without the blower (Pinter et al., 2000). These blower systems kept a constant CO₂ concentration inside the ring using an automatically controlled computer system based on wind speed, wind direction and CO₂ concentration inside the ring. Average 24-h CO₂ concentrations over two growing seasons measured from 10 cm above crop canopy in the center of each plot were 582 μmol mol⁻¹ and 399 μmol mol⁻¹ in FACE and Control, respectively. Each of the circular FACE and Control plots was also split in semicircular halves, with each half receiving either well-watered...
(Wet) or a water-limited (Dry) regime. Our research on soil respiration was only conducted in the wet treatment of replicates 1 and 2, so following description and analysis are focused on CO₂ effects under high soil moisture.

Level-basin flood irrigation was utilized to maintain soil-water content (θ) near field capacity in Wet plots. The criterion used to decide when and how much to irrigate was that after 30% of the available water in the rooted zone was depleted, the field was then irrigated with an amount calculated to replace 100% of the potential evapotranspiration since the last irrigation, adjusted for rainfall. The total amounts of irrigation plus rain applied during 1998 and 1999 were 1218 mm and 1047 mm, respectively, in both FACE and Control wet plots. Prior to planting, the field was laser-leveled and disked in two directions, and the first fertilizer was applied by air at a rate of 93 kg N ha⁻¹ and 41 kg P ha⁻¹. The second application of fertilizer was applied during the early growing season with irrigation at a rate of 186 kg N ha⁻¹ to give a total N application of 279 kg N ha⁻¹ and 266 kg N ha⁻¹, for the 1998 and 1999 season, respectively. The seed (Sorghum bicolor cv. Dekalb DK54) had been treated with fungicides, and was planted in north-south rows spaced 0.76 m (30 inches) apart at rate of 318000 seeds ha⁻¹ on 15-16 July in 1998 and on 14-15 June in 1999, one month earlier in 1999 than 1998. Heading and anthesis occurred from 27 September to 2 October in 1998 and from 24 to 29 August in 1999, and physiological maturity was about 23 November in 1998 and 27 September in 1999. Visually, there was no difference in phenology between FACE and Control treatments in either season. The final sorghum grain harvest for these two growing seasons was on 21 December in 1998 with a total 159 growth days and on 26 October 1999 with a total 133 growth days. Of course, the sorghum crop matured significantly
earlier in 1999 than 1998 because of the earlier planting and the subsequent warmer temperatures.

In addition to FACE and Control plots, we reserved a 4m by 4m fallow plot in the sorghum field for comparison. This fallow plot experienced the same plowing, fertilizing and irrigation as FACE and Control plots except sorghum plants were excluded. We also removed any weeds growing in this plot. In the second growing season the fallow plot received the same quantity of sorghum residue as FACE and Control. We assume soil respiration efflux from the fallow plot contains no root-affected decomposition of soil organic matter (SOM). Although there exists an inherent problem in taking fallow soil respiration rate as an indicator of old SOC decomposition rate in FACE or Control plots because SOM decomposition will be different with or without belowground root system and aboveground canopy, it is highly likely that respired CO$_2$ from fallow plots carries the precise isotopic signature of CO$_2$ derived from non-root-affected SOC degradation including pre-season litter decomposition and old soil humus breakdown. Therefore, using the isotopic signature of fallow respired CO$_2$ as an isotopic representative of old SOC decomposition to partition respired CO$_2$ is more precise than using soil humus isotopic value because the isotopic signature of respired CO$_2$ in the fallow plot has included isotopic disturbance from pre-season litter decomposition.

Soil Respiration Measurement

Soil respired CO$_2$ efflux was measured \textit{in situ} from the soil surface at the midpoint between sorghum crop rows biweekly during August-December of 1998 and July-September of 1999, using a portable Licor-6200 CO$_2$ analyzer and permanently installed
PVC (polyvinyl chloride) collars (Pendall et al., 2001). The cylindrical PVC collars, 14 cm (5.5 inch) high and 20 cm (7.9 inch) in diameter and open at both ends, were inserted into the soil to a depth of ~6 cm to ensure a good seal at the PVC collar-soil interface at the beginning of growing season, where they remained undisturbed throughout the growing season. There were three 2.6-cm-diameter holes around the middle of each PVC collar just at the ground level, which allowed irrigation water and air to flow through the collars freely and maintained the same micrometerological condition within collars as that outside between biweekly measurements. These three holes were carefully covered with rubber stoppers and the top of the collars were covered when soil respiration was measured.

Measurement of soil respiration was conducted by covering the PVC collar with the CO₂ sensor plate connected to the CO₂ analyzer of the Li-6200 portable system. It recorded the change in CO₂ concentration in the PVC collar every 4 and 10 seconds over 2-3 min. There were two duplicate PVC collars installed in each plot and two measurements were made for each collar on each sampling date. Therefore, there are 4 measurements for each plot and 8 replicate measurements for each treatment. All the measurements occurred in mornings from 09:00 to 12:00 noon local time (Mountain Standard) in the order of 1F, 1C, 2C, 2F and fallow. Respiration rate was calculated from CO₂ accumulation rates based on mean value obtained from 8 repeated measurements and expressed as µmol CO₂ m⁻² s⁻¹. The first soil respiration was measured at the date when 50% of the plants had emerged and the last measurement was made after sorghum entered physiological maturity. There were 8 measurement dates in 1998, and 7 in 1999. It should be pointed out that although the gas inside the PVC collar is a mixture of soil-
respired CO₂ and atmospheric CO₂ initially trapped in the PVC collar, this mixture slightly disturbs the determination of respired CO₂ isotope composition but does not affect the soil respiration rate after the PVC is covered with CO₂ sensor cap.

Respired CO₂ and Soil Air Sampling

In addition to CO₂ flux measurements, we collected the respired CO₂ immediately after respiration rate was recorded, using 125-ml double-stopcock flasks that were connected in line between CO₂ sensor and Li-6200 device through rubber tubing. The respired CO₂ samples were then brought to the laboratory for CO₂ and isotopic analysis. Soil air was sampled using evacuated 125-ml single-stopcock flasks similarly at biweekly intervals from depths of 15 cm and 30 cm through the buried tubes for CO₂ recovery and isotopic analysis. There were four perforated stainless steel tubes (12 cm long and 20 mm-diameter) separately connected with 4-mm-diameter plastic tubing at one end and horizontally emplaced in the soil at 15 cm and 30 cm depths (two duplicate tubes at each depth) just below the sorghum crop row immediately after sorghum seeds were planted. The open end of the plastic tubing was left at the ground surface and sealed with silicone sealant between sampling to avoid atmospheric air directly exchanging with deep soil air through the tubing. When soil air CO₂ was sampled, the sealing silicone was removed temporarily and pre-evacuated 125-ml flasks was attached to the tubing with an o-ring compression connector. Before opening the flasks, 50-ml of air within the tube was withdrawn with a syringe to remove any atmospheric air contamination from the tube followed by 90 seconds to restore the balance of soil air in the tube and soil. After carefully and slowly opening the flask stopcocks, soil air in the tube was inhaled into the
flask, another 90 seconds was allowed to regain the balance of the soil air in flask and tube, and the flask stopcock was then closed. Soil air samples were collected on 10 occasion in 1998 and 8 in the 1999 growing season.

**Stable Carbon Isotope Analysis**

Respired CO₂ and soil air samples were brought to laboratory for purification and isotopic composition analyses. Each sample was first admitted sequentially into one cold (-80 °C) ethanol trap and two liquid N₂ traps on a vacuum line, allowed to stand in traps for 5 minutes to ensure that all water was trapped in ethanol trap and that CO₂ was trapped into the liquid N₂ traps. The gas stream was slowly bled through the traps until other gases were completely removed and the line was evacuated. CO₂ trapped in the two liquid N₂ traps was vaporized by heating and re-trapped from the line into 10-ml vials with liquid N₂. This procedure separates pure CO₂ from H₂O and other gases in the sample without fractionating the carbon isotopes. The yield of the purified CO₂ was measured with a manometer on a vacuum line before running the samples on a Finnigan Delta-S mass spectrometer for isotope ratios. The stable isotopic composition of the purified CO₂ was reported as δ¹³C (‰) = (R_{sample}/R_{std}-1)* 1000 with PDB as a standard (where R_{sample} and R_{std} are ¹³C/¹²C for the sample and standard). The isotopic precision of the mass spectrometer is estimated to be 0.1‰ based on repeated measurements of several standards.

Prior to planting sorghum, four replicates of soil samples were collected at three depths of 0-15, 15-30 and 30-60 cm from both FACE and Control plots. Air above the canopy in each plot and pure tank CO₂ was regularly collected during the growing
season. Whole sorghum plant matter including leaf, stem and root and the three depths of soil were also sampled at the end of growing season for isotopic analyses. Air samples were processed in the laboratory the same as soil gas and respired CO$_2$. The soil samples were air-dried and sieved through 2 mm to remove pebbles and plant fragments. About 30 g of subsample was treated with 1 N HCl to remove any carbonates for C isotopic analyses (see detail procedure in Leavitt et al., (in revision). Sorghum samples were dried at 70 °C oven for 48 hours and ground to pass through a 40-mesh sieve. The ground sorghum tissues and the carbonate-free soil were combusted at 900 °C for 2 hours and then switched to 650 °C for another 2 hours in the presence of CuO, and the purified CO$_2$ from these combustions was collected similarly on the vacuum line. The C isotopic composition of the purified CO$_2$ from above-canopy air, sorghum plants and soil was determined using the Finnigan Delta-S mass spectrometer.

**Respired CO$_2$ Partitioning**

We employed a $^{13}$C-enriched commercial tank CO$_2$ derived from a geologic deposit with $\delta^{13}$C $\approx$ -4‰. Current atmospheric CO$_2$ has an isotopic composition with $\delta^{13}$C $\approx$ -8‰. Air in Control plots derives from the atmosphere with an atmospheric isotopic composition, resulting in Control C$_4$-sorghum plants with $\delta^{13}$C approaching $\approx$ 11.6‰. Air in FACE plots was composed of background air plus tank CO$_2$, resulting in its isotopic composition being about $\approx$ -7.6‰. Consequently, $\delta^{13}$C of sorghum in FACE plot is $\approx$ -10.4‰, 1.2‰ less negative than that of Control sorghum. Soil organic matter in the field was mostly derived from past-cultivated C$_3$ plants (e.g., cotton, wheat) although the last crop immediately prior to the experiment was C$_4$ sorghum. C$_3$ plants have a
photosynthetic pathway favoring incorporating of $^{12}$C that is different from the C$_4$ pathway, which discriminates much less $^{13}$C. These different photosynthetic pathways resulted in C$_3$ plants being much more depleted in $^{13}$C than C$_4$ plants and therefore the isotopic composition of soil organic matter at the beginning of the experiment is homogeneously with $\delta^{13}$C=-22.65‰. (See Table 1---Isotopic composition of FACE sorghum experiment system). Significant difference in isotope composition of the C sources in our FACE experiment provides a helpful isotopic tracer for partitioning respired CO$_2$.

Based on different isotopic composition caused by different C sources, respired CO$_2$ was quantitatively partitioned into root-respired CO$_2$ and old SOC degraded CO$_2$, using the following equation.

$$f_{\text{root}} = \frac{\delta^{13} C_{\text{sample}} - \delta^{13} C_{\text{SOC}}}{\delta^{13} C_{\text{root}} - \delta^{13} C_{\text{SOC}}}$$

$$f_{\text{soc}} = 1 - f_{\text{root}}$$

Where, $f_{\text{root}}$ and $f_{\text{soc}}$ are the proportions of CO$_2$ generated by root activity including living root respiration and microbial degradation of newly formed material from live roots and pre-label SOC degradation, respectively. $\delta^{13}$C$_{\text{sample}}$, $\delta^{13}$C$_{\text{soc}}$ and $\delta^{13}$C$_{\text{root}}$ are $\delta^{13}$C values of respired CO$_2$, pre-label SOC and root-activity-produced CO$_2$, respectively. In this equation, we assume the respired CO$_2$ originated from two sources. Actually, it came from various soil C pools with various isotopic compositions. To properly partition respired CO$_2$ into two parts, we assume that, beside the $\delta^{13}$C value of the root-activity-
Table 1. Isotopic composition in FACE sorghum experiment system. Parameter values are expressed in Mean±standard deviation and n is the number of samples. Atmospheric CO₂ concentration in the unit of µmol mol⁻¹ is a daily average of the value measured during growth season. Sorghum samples were collected from four replicate plots at each end of growth season and isotopic δ¹³C values were measured from whole sorghum tissues. Soil organic C content and isotopic composition shown here were analyzed from the 0-15cm soil samples collected at the beginning of the experiment in 1998 and at the end of experiment in 1999.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>FACE 1998</th>
<th>FACE 1999</th>
<th>Control 1998</th>
<th>Control 1999</th>
<th>Difference (F-C) 1998</th>
<th>Difference (F-C) 1999</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atmospheric CO₂: Concentration (µmol mol⁻¹)</td>
<td>556</td>
<td>566</td>
<td>363</td>
<td>373</td>
<td>+193</td>
<td>+193</td>
</tr>
<tr>
<td>Isotopic δ¹³C (%)</td>
<td>-7.56±0.46 n=33</td>
<td>-7.63±0.51 n=18</td>
<td>-8.93±0.64 n=23</td>
<td>-9.42±0.80 n=19</td>
<td>+1.37</td>
<td>+1.79</td>
</tr>
<tr>
<td>Sorghum δ¹³C (%)</td>
<td>-10.39±0.19 n=4</td>
<td>-10.35±0.02 n=4</td>
<td>-11.36±0.05 n=4</td>
<td>-11.80±0.16 n=4</td>
<td>+0.97</td>
<td>+1.45</td>
</tr>
<tr>
<td>Soil Organic C*: Content (%)</td>
<td>0.69±0.08 n=8</td>
<td>0.72±0.06 n=8</td>
<td>0.72±0.11 n=8</td>
<td>0.67±0.05 n=8</td>
<td>-0.03</td>
<td>+0.05</td>
</tr>
<tr>
<td>Isotopic δ¹³C (%)</td>
<td>-22.61±0.32 n=8</td>
<td>-20.91±0.70 n=8</td>
<td>-22.68±0.37 n=8</td>
<td>-21.76±0.36 n=8</td>
<td>+0.07</td>
<td>+0.85</td>
</tr>
</tbody>
</table>

* 2 samples from each of 4 replicates
produced CO₂ that represented CO₂ derived from new C sources was assumed to be equal
to average sorghum δ¹³C value, the δ¹³C value of pre-label SOC in the equation was
replaced by the δ¹³C of fallow soil air in depth of 15 cm instead of using the isotopic
composition of SOC collected in the field before experiment. By doing so, we classified
the rest of respired CO₂ coming from new organic carbon as same C pools, which include
SOC decomposition and non-SOC (e.g. pre-seasonal litter and other soil macro
organisms) decomposition. Because of CO₂ δ¹³C in soil respiration possibly being
contaminated by the mixture with atmospheric air, which is difficult to determine because
local microclimate factors controlling this mixture vary from each measurement (Cerling,
1991; Dorr and Munnich, 1980), the δ¹³C value of soil CO₂ in 15 cm depth was supposed
to have minor effects by atmospheric CO₂ and was used to represent the isotopic
signature of real respired CO₂.

It should be pointed out that soil air CO₂ δ¹³C values are more enriched in ¹³C
than the organic matter from which they are derived. This fractionation is caused by the
difference of the diffusivities of the two isotopic species (the lesser diffusivity of ¹³CO₂
relative to ¹²CO₂). At the steady-state condition, the diffusive fractionation is eliminated
by the imposition of mass balance and the δ¹³C value of soil gas reservoir approaches a
value ~ 4.4‰ higher than the production (Amundson et al., 1998; Cerling, 1984).
Therefore, prior to using the above equation to partition respired CO₂, the δ¹³C value of
soil air sample was corrected by subtracting 4.4‰ for this kinetic fractionation produced
during diffusion. Because, at steady state, the δ¹³C value of the respired CO₂ is equal to
that of biologically produced CO₂, this provides us a reason to assume that the portion of
root respired CO₂ or native SOC degraded CO₂ in soil gas is the same as that in respired
air, and that there is no fractionation of root respired CO$_2$ and SOC degraded CO$_2$ during the sorghum growing season. Based on this assumption, portions of root respired CO$_2$ and native SOC degraded CO$_2$ in soil air was assumed to reflect the portions in respired CO$_2$.

Statistical analyses

Studies in specific ecosystems indicate that soil respiration is influenced by various factors, such as soil water content, temperature, fertility and soil surface micrometeorological factors. In our FACE experiment, these factors are maintained at comparable levels between FACE and Control plots and only CO$_2$ concentration is varied. Therefore, only one-way ANOVA and paired-t test were used to test the significant difference of CO$_2$ effects on total respired CO$_2$ and the partitioned CO$_2$ derived from root respiration or from native SOC decomposition.

Results

Seasonal CO$_2$ flux patterns and soil air CO$_2$ concentration

FACE significantly increased soil respiration rate in both years, but the 1998 seasonal pattern was different than 1999 (Fig. 1a, b). The seasonal mean soil respiration rate measured in elevated CO$_2$ plots over two growing seasons was 3.3 µmol m$^{-2}$ s$^{-1}$, 12.7% higher than the 2.9 µmol m$^{-2}$ s$^{-1}$ measured in ambient CO$_2$ plots (Table 2). Increased CO$_2$ flux in FACE was much greater in 1998 (41.9%) than in 1999 (2.3%). As shown in Figure 1, elevated CO$_2$ resulted in significantly higher soil CO$_2$ flux in FACE plots throughout growing season in 1998 with higher flux (5.5 µmol m$^{-2}$ s$^{-1}$) during the
period of active vegetative growth and lower flux (1.3 μmol m\(^{-2}\) s\(^{-1}\)) during physiological maturity. However, in 1999 elevated CO\(_2\) had a smaller effect on soil CO\(_2\) flux with FACE CO\(_2\) flux slightly lower than Control before sorghum heading and higher during the late growing stages.

Because fluxed CO\(_2\) comes from soil air, elevated CO\(_2\) effects on the CO\(_2\) flux should also be expressed in soil air CO\(_2\) concentrations. Based on calculations with recovered CO\(_2\) collected from the soil profile, FACE significantly increased soil CO\(_2\) concentration by 18.2% over two growing seasons. For both FACE and Control treatments, the amount of \textit{in situ} CO\(_2\) decreased during the growing season. The rapid decline during early growth season may have resulted from high CO\(_2\) losses related to the early tillage, but the decline in all treatments over the remainder of the growing season may reflect decreasing microbial activity and/or root activity. Compared with Control, the increased FACE soil CO\(_2\) concentration was much greater in 1998 (35.6%) than in 1999 (3.7%), which coincides with CO\(_2\) flux comparisons. These measurements reveal a strong seasonality in soil CO\(_2\) concentration similar to that in soil respiration, being low (500-1000 μmol mol\(^{-1}\)) in the physiological maturity stage and high (15,000-30,000 μmol mol\(^{-1}\)) during the active vegetative growing stage. Spatially, soil CO\(_2\) concentration was much higher at 30 cm than 15 cm, but in 1998 FACE enhancement was higher (35.6%) at 15 cm with less variability and lower (32.8%) at 30 cm with large variability. In 1999, however, the increase in FACE soil CO\(_2\) concentration was only 3.70% at 15 cm and 5.05% at 30 cm. (Fig 1c, d, and Table 2)
Table 2. Mean±standard deviation of resulting parameters across both sorghum growth seasons under elevated (“FACE”: 560 µmol mol⁻¹) and ambient (“Control”: 360µmol mol⁻¹) CO₂ conditions. Also shown are the FACE enhancements based on Control.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Year</th>
<th>FACE</th>
<th>Control</th>
<th>FACE Enhancement Based on Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil respiration rate (mol CO₂m⁻²s⁻¹)</td>
<td>1998</td>
<td>1999 Mean</td>
<td>2.19±0.51 4.41±0.57 3.30</td>
<td>1.54±0.34 4.31±0.73 2.93</td>
</tr>
<tr>
<td>Respired CO2 isotopic δ¹³C (‰)</td>
<td>1998</td>
<td>1999 Mean</td>
<td>-10.79±0.47 -11.23±0.53 -11.01</td>
<td>-13.23±0.49 -12.30±0.55 -12.77</td>
</tr>
<tr>
<td>Root respiration rate (mol CO₂m⁻²s⁻¹)</td>
<td>1998</td>
<td>1999 Mean</td>
<td>0.85±0.24 1.69±0.25 1.27</td>
<td>0.50±0.13 1.37±0.15 0.93</td>
</tr>
<tr>
<td>SOC decomposition rate (mol CO₂m⁻²s⁻¹)</td>
<td>1998</td>
<td>1999 Mean</td>
<td>1.34±0.28 2.72±0.66 2.03</td>
<td>1.05±0.21 2.94±0.74 1.99</td>
</tr>
<tr>
<td>Proportion of root respired CO₂, fᵢ</td>
<td>1998</td>
<td>1999 Mean</td>
<td>0.395±0.044 0.513±0.041 0.454</td>
<td>0.329±0.037 0.449±0.047 0.389</td>
</tr>
<tr>
<td>Proportion of SOC degraded CO₂, fᵢ</td>
<td>1998</td>
<td>1999 Mean</td>
<td>0.606±0.044 0.488±0.041 0.547</td>
<td>0.671±0.037 0.551±0.047 0.611</td>
</tr>
<tr>
<td>Recovered soil [CO₂] (µmol mol⁻¹)</td>
<td>1998</td>
<td>1999 Mean</td>
<td>15799±2185 13806±2223 14802</td>
<td>11793±1727 13217±2714 12505</td>
</tr>
<tr>
<td>Recovered soil CO₂ isotopic δ¹³C (‰)</td>
<td>1998</td>
<td>1999 Mean</td>
<td>-14.79±0.92 -11.92±0.90 -13.35</td>
<td>-15.47±0.84 -12.78±0.65 -14.12</td>
</tr>
<tr>
<td>Root respired CO2 in soil air (µmol mol⁻¹)</td>
<td>1998</td>
<td>1999 Mean</td>
<td>4776±752 6769±840 5772</td>
<td>3225±479 5338±901 4281</td>
</tr>
<tr>
<td>SOC degraded CO2 in soil air (µmol mol⁻¹)</td>
<td>1998</td>
<td>1999 Mean</td>
<td>11024±2237 7037±1741 9030</td>
<td>8568±1717 7880±2380 8224</td>
</tr>
</tbody>
</table>
Fig. 1. Seasonal soil respiration rates (mol CO$_2$ m$^{-2}$ sec$^{-1}$) of sorghum FACE and Control during 1998 (a) and 1999 (b), and soil CO$_2$ concentration (µmol mol$^{-1}$) measured from FACE, Control, Fallow plots at the depths of 15 cm and 30cm in 1998 (c) and 1999 (d) growing seasons. These figures showed that FACE significantly increased both soil respiration by 12.7% and soil air CO$_2$ concentration by 18.4% over two growing seasons, but the 1998 seasonal patterns were different from 1999’s. Enhancements were much greater in 1998 than 1999.
\( \delta^{13}C \) Variation in respired \( CO_2 \) and soil air \( CO_2 \)

The averaged \( \delta^{13}C \) values of FACE respired \( CO_2 \) were less negative than the Control plots by 2.44‰ in 1998 and by 1.07‰ in 1999 (Fig. 2a and b; Table 2). These differences could be caused by higher FACE sorghum root activity or possible mixture of respired \( CO_2 \) with FACE atmospheric \( CO_2 \), which has less negative \( \delta^{13}C \) than Control. Atmospheric air disturbance cannot be neglected, especially at the beginning of growing season when sorghum seedlings were shorter and sparse, and up to 70%-80% of atmospheric \( CO_2 \) estimated in the respired \( CO_2 \) (data not shown) with higher \( \delta^{13}C \) values measured early in the growing season. This mixture portion is difficult to accurately calculate when the exact isotopic composition of atmospheric \( CO_2 \) and respired \( CO_2 \) are not known. As sorghum grew, reducing the mixture-promoting microclimate factors (e.g. wind speed, temperature changes, \([CO_2]\) gradient at the soil surface, etc.), the atmospheric mixing was reduced and root respiration and heterotrophic activity would dominate the respired \( CO_2 \). These biological seasonal changes in \( \delta^{13}C \) values were observed after sorghum heading and anthesis, showing that the \( \delta^{13}C \) values of respired \( CO_2 \) slightly increased with sorghum growth and leveled off when sorghum growth reached maturity.

The \( \delta^{13}C \) variations of soil air \( CO_2 \) contain minor effects of atmospheric \( CO_2 \) mixture or diffusion (Andrews et al., 1999) and are dominated by sorghum root and soil heterotrophic activities. The average \( \delta^{13}C \) values of soil air \( CO_2 \) collected from FACE, Control and Fallow plots at depths of 15 cm and 30 cm were shown in Fig. 2c(1998) and 2d (1999). The patterns were similar in both years, with FACE soil \( CO_2 \) having less negative \( \delta^{13}C \) values than Control by 0.68‰ (1998) and 0.86‰ (1999), and both FACE
Fig. 2. Isotopic δ^{13}C variations of the soil respired CO₂ measured in FACE and Control plots during 1998 (a) and 1999 (b) growing seasons, and the soil air CO₂ measured from FACE, Control and Fallow at the depths of 15 cm and 30 cm in 1998 (c) and 199 (d) growing seasons. The figures show that the δ^{13}C values of the respired CO₂ in FACE plots were less negative than the Control plots by 2.44‰ in 1998 and by 1.07‰ in 1999, which may be caused by higher FACE sorghum root activity. δ^{13}C variation in soil air CO₂ much better reflects the elevated CO₂ effects on belowground processes because of minor influence of atmospheric air diffusion. Undoubtedly, the difference in soil air δ^{13}C between Fallow and FACE and Control was caused by root activity, and the difference between FACE and Control was resulted from elevated CO₂.
and Control having much higher $\delta^{13}$C than Fallow by 3.95‰ (1998) and 4.57‰ (1999). The isotopic differences between 15 cm and 30 cm for the same treatment did not appear significant. Seasonally, soil CO$_2$ $\delta^{13}$C of all treatments was lower with a close value of –22.23‰ (1998) and –18.52‰ (1999) early in the growing season, indicating soil air CO$_2$ was mainly derived from decomposition of pre-label soil organic matter, which was very depleted in $^{13}$C. The $\delta^{13}$C values increased rapidly in the vegetative growth stage until sorghum heading and anthesis signaled the rapid growth of sorghum plants and a significant portion of root respired CO$_2$ in the soil air. In later growing stages there was an inflection in the curve of $\delta^{13}$C values related to serious frost damage in 1998 (on 10-Nov.) and hail damage in 1999 (on 16-Sept.). Fallow plots also showed increase in $\delta^{13}$C values in the early season that resulted from the increased decomposition of pre-experiment C$_4$ sorghum residues, stimulated by early tillage, irrigation and fertilization. But the increase in soil CO$_2$ $\delta^{13}$C in the Fallow plot was much less and more graduated than in the FACE and Control plots, and during most of the experiment Fallow soil air had more negative $\delta^{13}$C values because of no root activity.

**Fraction variation of root-respired CO$_2$ and SOC-degraded CO$_2$**

As stated in the methods section, the soil air $\delta^{13}$C variation in Fallow plots can be considered to represent the isotopic signature of the CO$_2$ derived from decomposition of the native soil organic matter that was input prior to the growing season, and included old SOC and pre-season-input plant residue. Undoubtedly, the difference in soil air $\delta^{13}$C between Fallow and FACE and Control was caused by root activity, and the difference between FACE and Control resulted from the effect of elevated CO$_2$. We assume that
Day after Planting
Fig. 3. Calculated fraction \( (f_i) \) of the root-respired CO\(_2\) in FACE and Control plots at the depths of 15 cm and 30 cm during 1998 (a-15cm, c-30cm) and 1999 (b-15 cm, d-30cm), and the SOC-degraded CO\(_2\) in FACE and Control plots at the depths of 15 cm and 30 cm during 1998 (e-15cm, g-30cm) and 1999 (f-15cm, h-30cm). These figures indicate that elevated CO\(_2\) increased root respiration fraction and reduced SOC degraded CO\(_2\) fraction compared to Control. This tendency was consistent over two growing seasons.

root-respired CO\(_2\) carries the same isotopic signature as sorghum plant tissues and SOC degraded CO\(_2\) has the same isotopic signature as in Fallow soil air. Based on the equation 1, the proportional contributions, \( f_i \), of root-respired CO\(_2\) and SOC-degraded CO\(_2\) to total soil CO\(_2\) at the 15cm and 30 cm soil air are shown in Fig. 3.

Overall, the seasonal patterns of root respired CO\(_2\) and SOC degraded CO\(_2\) proportions were similar in the two growing seasons, with FACE having higher root-respired CO\(_2\) and lower SOC degraded CO\(_2\) proportions than Control, and root-respired CO\(_2\) proportion increasing and SOC-degraded CO\(_2\) proportion decreasing as the growing season progressed. The main differences between two seasons were that (1) during the rapid growth stage from heading to physiological maturity, root-respired CO\(_2\) proportions were 39.4% in FACE and 32.9% in Control in 1998, lower than 53.6% (FACE) and 48.1% (Control) in 1999, and (2) SOC-degraded CO\(_2\) proportions were 60.6% (FACE) and 67.2% (Control) in 1998, higher than 46.5% (FACE) and 51.9% (Control) in 1999. These data indicate although the portion of root-respired CO\(_2\) in soil air in 1999 was higher than 1998, FACE enhancement of root respiration compared with Control was higher in 1998 than 1999. Spatially, there were minor differences in SOC decomposition...
between the depths of 15 cm and 30 cm for same treatment, with FACE enhancement of root respiration slightly higher at 30 cm than 15 cm.

Calculated on the basis of the above proportions, the concentration of root-respired CO$_2$ and SOC-degraded CO$_2$ in soil gas is shown in Fig. 4. Elevated CO$_2$ significantly increased root respiration, and the enhancement is much higher in 1998 than 1999. FACE root respiration averaged 52.4% at 15 cm and 44.8% at 30 cm higher than control in 1998, and 22.4% at 15 cm and 30.2% at 30 cm higher than control in 1999. The effect of elevated CO$_2$ on old SOC decomposition, however, was not consistent over two growing seasons. Elevated CO$_2$ increased old SOC decomposition by 28.7% in 1998, whereas it decreased by 10.5% in 1999. It does not seem to support the hypothesis that higher root respiration will be associated with higher SOC mineralization (Zak et al., 1993). Also, we found no significant correlation between root respiration and old SOC decomposition ($r=0.014$ in 1998 and $r=0.163$ in 1999). Seasonally, compared to Control, FACE root respiration increased more rapidly in the early growing season, reached a peak at the heading and anthesis period and then decreased, whereas old SOC decomposition decreased from the beginning to end of the growing season. High SOC decomposition was observed at the beginning of growing season because the activities of tillage, irrigation and fertilization just before planting, offered favorable conditions for heterotrophic activity, and also because the previous year’s residues and native humus steadily supplied significant amounts of carbon. The decreased tendency of SOC decomposition also indicates that the role of the native soil carbon decreased with sorghum growth when current year’s residues became available.
Day after Planting
Fig. 4. Estimated root respired CO₂ concentration (µmol mol⁻¹) in FACE and Control soils at the depths of 15 cm and 30 cm during 1998 (a-15cm, c-30cm) and 1999 (b-15cm, d-30cm), and estimated SOC-degraded CO₂ concentration (µmol mol⁻¹) in FACE and Control soils at the depths of 15 cm and 30 cm during 1998 (e-15cm, g-30cm) and 1999 (f-15cm, h-30cm). The figures showed that FACE had higher root respiration by 16.7% and lower SOC decomposition by 10.5% than Control. Compared over two growing seasons, FACE increased root respiration by an average of 34.8%, but the effect on old SOC decomposition was found significant difference from Control only in 1998.

Discussion

Researchers studying C₃ plants have proposed that stimulation of soil respiration by elevated CO₂ occurs as a consequence of higher plant photosynthetic rates, which lead to higher plant metabolism and hence, higher root respiration, inducing enhanced SOM decomposition (Fontaine et al., 2003; Sadowsky and Schortemeyer, 1997). These effects should be minor in systems where C₄ plants are growing because no large enhancement of photosynthesis is expected (Lambers, 1996). However, our results with C₄ sorghum show that FACE significantly increased soil respiration over two growing seasons, especially in 1998 in which soil respiration in FACE increased by 42.2% comparing with Control (Fig. 1). This indicates that either root or microbial activity is increased in the soil profile, which should be initially stimulated by the enhancement of sorghum growth in elevated CO₂. Although the response of C₃ and C₄ plants to elevated CO₂ differ, well-watered C₄ plants often exhibit enhanced net assimilation, increased growth, and disproportional increases in C allocation to roots under elevated CO₂ condition (Wand et
Ottman et al. (2001) reported that sorghum stover in our FACE experiment increased by 11% and 3% in 1998 and 1999, respectively, although total biomass aboveground only increased in 1998 and was not significantly different in 1999. Additionally, Wall et al. (2001) reported that FACE increased sorghum water-use efficiency, which should increase soil water availability in elevated CO2 plots and may possibly amplify CO2 effects on soil respiration through enhanced plant growth and positive feedback on microbial activity.

Effects of elevated CO2 on soil respiration vary greatly not only for different species but also for various environmental factors (Lomander et al., 1998). The seasonal variation of soil respiration in our research did not exhibit the same pattern over both growing seasons (Fig. 1). It was probably caused by different growing periods---one month earlier planting in 1999 than 1998. Consequently, meteorological conditions differed between growing seasons even through we tried to keep other factors such as soil moisture, soil fertilization and CO2 concentration equal. Modification of the effect of elevated CO2 on soil respiration by other environmental factors has been reported for *Deschampsia flexuosa* and *Holcus lanatus* (Poorter et al., 1995), *Pseudotsuga menziesii* (Lin et al., 2001), and *Bromus hordeacus* grassland (Luo et al., 1996). However, no matter how modified, the variation of soil respiration was caused by the changes in root respiration, SOM decomposition, and their interaction in the soil profile.

In our research, the effects of elevated CO2 on root respiration were relatively consistent. Higher root respiration in FACE plots was observed during both years of the experiment. This could be a consequence of increasing root turnover and exudation, resulting from increased photosynthesis under elevated CO2. However, the CO2-
stimulated seasonal average soil carbon efflux was 30% and 28% in 1998 and 1999, respectively (Table 2), which is much more than the increase in aboveground biomass (Ottman et al., 2001). This indicates that a considerable amount of carbon was translocated below ground for root respiration, root turnover, and root exudation. The supply of labile carbon from root exudation, sloughed-off material and dead roots in the soil is reported significantly elevated under enhanced CO₂, increasing the total carbon allocated below-ground in the form of roots and root-derived products (Bille et al., 1993; Lekkerkerk et al., 1990; Norby et al., 1987; Rouhier et al., 1994). Additionally, Pregitzer et al. (1995) and Berntson (1996) have found that the total amount of root senescence is greater with elevated CO₂. Pregitzer et al. (1995) suggested that this increase was the result of reduced survivorship of individual roots. Berntson and Bazzaz (1996b) found that root production and root loss were highly correlated, and that increase in the rate and amount of total root loss in elevated CO₂ appeared to be driven by increases in root production.

We also observed that root respiration was more enhanced at 30-cm depth, which implies that root architecture (morphology and spatial pattern of root deployment) may be altered by elevated CO₂. Rogers et al. (1994a; 1994b) reported that elevated CO₂ resulted in root systems having greater total root length, penetrating deeper into the soil and producing more root tips. This is because an increase in photosynthesis at elevated CO₂ increases the pool of total nonstructural carbohydrates (Drake et al., 1997; Estiarte et al., 1999) that can be used to develop a more robust root system (Rogers et al., 1992; Wechsung et al., 1999). Chaudhuri et al. (1986) demonstrated that elevated CO₂ increased root mass at every growth stage of sorghum, and that roots reached the bottom
of a 1.6-m mini-rhizotron faster under elevated than ambient CO$_2$. A corresponding increase in root length and density was also observed, which suggest alterations in morphological characteristics (increased branching and number of fine roots) in sorghum grown in elevated CO$_2$ (Chaudhuri et al., 1986), as observed in other species (Rogers et al., 1992).

Seemingly, our results do not strongly support the hypothesis that increased plant root respiration will be accompanied by an increased SOM mineralization rate (Hungate et al., 1997). Although FACE root respiration was significantly increased in both years, SOC mineralization rate was increased only in the first year by 28.7% and was significantly decreased in the second year by 10.5%. This also conflicted with the findings from prior FACE cotton (Nakayama et al., 1994) and wheat (Pendall et al., 2001) experiments in which SOC decomposition was significantly stimulated by increasing root respiration. There are several reported hypotheses to explain lower SOC decomposition under elevated CO$_2$. One is ‘high CO$_2$ suppression’ that suggests that SOM decomposition can be temporally suppressed by increased CO$_2$ concentration in soil air (Santruckova and Simek, 1997). Another is ‘preferential substrate utilization’ that states that soil microorganisms prefer labile root-derived C to soil-derived carbon, and as a result, there is decreased SOM decomposition when more root-derived C is produced in elevated CO$_2$ (Kuikman et al., 1990). A third is ‘old SOM immobilization’ that suggests that extra C alters soil microbial processes by providing needed substrates, thereby suppressing SOM decomposition resulting from microbial immobilization (Diaz et al., 1993). The last is the ‘competition’ hypothesis that states there is competition for mineral nutrients between plants and soil microorganisms. Enhancement of plant growth by
elevated CO₂ results in reducing the mineral nutrient available to soil organisms, thereby decreasing SOM decomposition (Ehrenfeld et al., 1997; Schimel et al., 1989). None of these hypotheses can clearly explain our results with stimulating SOC decomposition in the first year and suppressing it in the second year. In our research, FACE increased soil CO₂ concentration only in 1998 with no significant effect in 1999. Therefore, there is insufficient information to support elevated CO₂ enhancing the soil CO₂ concentration to an extent that is likely to affect SOC decomposition in our research. Nutrient competition is also highly unlikely in our FACE experiment with abundant fertilization.

From our results, we hypothesize that FACE stimulation of SOC decomposition is primarily within the SOC in the active carbon pool with short turnover time. This implies that stimulation of SOC decomposition by elevated CO₂ depends on the SOC quality and operates on a short-term basis. Once the organic matter in the active C pool is degraded, stimulation of decomposition will be greatly reduced. Particularly in the agroecosystems with abundant mineral nutrient supply, temporal stimulation of soil microbial growth/activity by adding labile C from roots will result in the immobilization of soil organic matter and eventually result in SOC having longer residence time with lower decomposition rates. Rillig et al. (2001) found that arbuscular mycorrhizal hyphal lengths and water-stable aggregates in our FACE soil significantly increased in response to elevated CO₂, demonstrating a great increase in soil microbial biomass.

Overall, our results showed the general increase in in situ soil respiration in a C₄-sorghum system exposed to elevated [CO₂], which agrees with previous findings based on other C₃ ecosystems (e.g. cotton (Nakayama et al., 1994) and wheat (Pendall et al., 2001) and supports the hypothesis of Zak et al. (1993) that stimulation of soil processes
occurs as a consequence of the effects of elevated [CO₂] on plant physiological processes. CO₂ in the enhanced respiration mainly (90%) derived from elevated rhizospheric respiration. Elevated CO₂ significantly increased root respiration during two growing seasons, indicating root metabolism, root exudates and root turnover was stimulated under elevated CO₂. Compared with the increase in sorghum plant growth, the CO₂-stimulated increase in root respiration is much higher than sorghum aboveground biomass. This suggests that most of the photosynthesis fixed C stimulated by elevated CO₂ will transfer to soil through the root system, and also suggests that root respiration can dissipate most of this C. In our experiment, the effects of elevated CO₂ on old SOC decomposition were inconsistent and the stimulation of SOC decomposition only occurred in the first year, which indicated that the “priming effect” is short-term behavior and appears to depend on SOC quality. However, there is currently no available information on the possible mechanisms of how SOC changes in quality and increases in mean turnover time under elevated CO₂, and this warrants further attention.

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APPENDIX B

Biochemical Compositions of Sorghum (*Sorghum Bicolor* (L.) Möench) Tissues in Response to Elevated Atmospheric CO$_2$ with Adequate and Deficient Water Supply

Abstract

Sorghum (Sorghum bicolor (L.) Möench) residue biochemical compositions resulting from elevated CO$_2$ with adequate and deficient water supply were studied in a Free-Air CO$_2$ Enrichment (FACE) experiment during 1998-1999 growing seasons at Maricopa, Arizona, USA. Sorghum samples were collected from FACE (560 µmol mol$^{-1}$ [CO$_2$]) wet (water-adequate) and dry (water-deficient) and Control (ambient 360 µmol mol$^{-1}$ [CO$_2$]) wet and dry treatments at the end of 1999 growth season. Sorghum leaf, stem and root composition was analyzed for amino acids (AAs), uronic acids (UAs), hemicellulose carbohydrates (HCCs), cellulose carbohydrates (CCs), lignin, non-lignin phenolics (NLPs), and C:N ratio. The results showed that elevated CO$_2$ and water-deficient treatments changed sorghum tissue biochemical compositions. On average, sorghum grown under elevated CO$_2$ reduced AAs and HCCs by 7% and 8%, respectively, whereas CCs and lignin were increased by 40% and 5%, respectively. Non-lignin phenolic acids were significantly higher in FACE roots, but no significant difference emerged between FACE leaves and stems and their control counterparts. Soil water stress largely enhanced the CO$_2$ effect on sorghum biochemical composition. For UAs, elevated-CO$_2$ effects were modified by soil moisture condition with a 7% reduction of UAs in FW and up to 6% increase in FD sorghum residues. The C:N ratio of sorghum tissues was not affected by CO$_2$, but was substantially lower under water stress. Our results indicated that CO$_2$-induced sorghum residue biochemical change may have significant effects on residue decomposition of sorghum grown under future CO$_2$-elevated environmental condition.
Key words: Elevated CO₂; FACE; Sorghum; Biochemical composition

Abbreviation: FACE---Free air CO₂ enrichment; AAs---amino acids; UAs--- uronic acids; HCCs--- hemicellulose carbohydrates; CCs--- cellulose carbohydrates; NLPs--- non-lignin phenolics; CBSSCs--- carbon-based secondary and structural compounds; TNC---total non-structural carbohydrates; NUE---nitrogen use efficiency; FD---FACE dry; FW---FACE wet; CD: Control dry; CW---Control wet.

Introduction

Atmospheric CO₂ concentration is expected to increase to more than 500 µmol mol⁻¹ by the end of this century and to have a profound effect on terrestrial ecosystems (IPCC (2001). However, there is still uncertainty whether the extent terrestrial ecosystem will act as a source or sink for elevated CO₂. Although many researchers have widely reported that elevated CO₂ increased terrestrial plant growth and biomass through increased plant photosynthesis, the effect of elevated CO₂ on plant quality is still poorly understood. Plant quality plays an important role in controlling residue decomposition, and subsequently determines carbon (C) fate in terrestrial ecosystems. Some researchers have proposed that terrestrial ecosystem would act as a sink of the atmospheric CO₂ because increased biomass production enhances soil organic material input, and consequently increases organic material storage in soil system (Bazzaz, 1990; Leavitt et al., 2001; Watson et al., 1990). Others, however, suggested that terrestrial ecosystems might act as a net source of atmospheric CO₂ because of a ‘priming effect’ whereby the input of new soil organic material stimulates microbial activity and the decomposition of old soil organic material, resulting in more CO₂ released from the soil ecosystem.
The resolution of this uncertainty may depend on how CO$_2$ affects the quality of plant residues formed under elevated CO$_2$ condition. The operation of a “priming effect” will be related to both the quantity of new organic material input, and the chemical composition of input organic materials.

Studies on the effect of elevated CO$_2$ on plant biochemical composition, however, are few in number and results thus far show that plant quality response to elevated CO$_2$ varies among plant species, environmental conditions, and growth stages. In general, plants grown under elevated CO$_2$ often, but not invariably, exhibit increased total nonstructural carbohydrate contents, C-based secondary compounds, and reduced N concentration, with a concomitant increase in C/N ratio ((Lambers, 1993; Peñuelas and Estiarte, 1998). Roumet et al. (1999) reported total non-structural carbohydrate (TNC) was 16.8% and 29.4% higher at elevated CO$_2$ than at ambient CO$_2$ for $B$. erectus and $D$. glomerata, respectively. Peñuelas et al. (2002) reported that whereas foliar total nonstructural carbohydrates concentrations were 13% greater in $Myrtus$ communis, they were 12% lower in $Juniperus$ communis in response to high CO$_2$. For the studied species, plant biochemical composition was more sensitive to elevated CO$_2$ at an early growth stage than in the mature stage. Larigauderie et.al. (1988) observed that $Bromus$ mothis leaves had a higher C:N mass ratio for the first 2 months of growth in an elevated atmospheric [CO$_2$], but that the CO$_2$ effect was not present in mature leaves at day 130. Similarly, for the grapevine studied by Bindi et al. (2001), substantial CO$_2$ effects on grape acid and sugar concentrations in the middle stages of the growth season were largely reduced after the plant matured. In addition, it was also observed that different plant tissues have a different composition response to elevated CO$_2$. For example, Booker
et al. (2000) reported that lignin concentrations in leaves and stems of cotton were not significantly affected by elevated CO$_2$ (722 µmol mol$^{-1}$), whereas root residue lignin concentration was 30% greater than that of the cotton grown in ambient CO$_2$. The change in biochemical composition for different plant parts plays a crucial role in the carbon cycle of terrestrial ecosystem, especially for roots that comprise nearly half the plant biomass and directly input new organic matter to soil.

To explain and predict the effect of elevated CO$_2$ on plant biochemical composition, several hypotheses have been proposed. The source-sink hypothesis assumes that elevated CO$_2$ concentrations promote a relative increase of carbon availability that is accumulated in total nonstructural carbohydrates and carbon-based secondary and structural compounds (CBSSCs) when the provided carbon amounts exceed growth requirements (Herms and Mattson, 1992). The carbon-nutrient balance hypothesis predicts that plants growing under elevated CO$_2$ have an ‘excess’ of carbon relative to the nutrients available for growth, and use it to produce terpenes, phenolics and other carbon-based compounds (Bryant et al., 1983; Hamilton et al., 2001). These hypotheses thus predict that increased carbon supply should increase concentrations of CBSSCs in plants grown under elevated CO$_2$ conditions. Many investigators have found increases in plant tissue production of secondary compounds in response to elevated CO$_2$ with C$_3$ species (Entry et al., 1998; Poorter et al., 1997) in apparent support of these hypotheses. However, some results from other studies are not consistent with these hypotheses. For example, Peñuelas et al. (2002) reported that phenolic concentrations in *Erica arborea* foliage were 28% lower under high CO$_2$ conditions near a CO$_2$ spring site than in the low CO$_2$ control site.
Sorghum, a C₄ plant, is expected to be less responsive to CO₂ than C₃ plant (Lawlor and Mitchell, 1991; Poorter, 1993; Poorter et al., 1997; Wand et al., 1999), because within the bundle sheath cells of C₄ plants CO₂ levels are near saturation and photorespiration is suppressed (Hatch, 1992; Nobel, 1991). However, elevated CO₂ has been reported to modify C₄ plant physiological processes, e.g. increasing net assimilation rate (Wand et al., 1999; Ziska et al., 1999), reducing stomatal conductance, and increasing water-use efficiency (Wall et al., 2001). Any modification in plant biochemical composition caused by elevated CO₂ will most likely occur because of improvements in such plant physiological processes.

The effects of elevated atmospheric CO₂ on ecosystems will not occur in isolation, but in the context of multiple environmental changes. In the future elevated CO₂ environment, terrestrial ecosystems are likely to face more severe drought conditions because of increased mean temperature and potential evapotranspiration. Soil moisture conditions will play an important role in directly modifying plant photosynthetic responses to elevated CO₂ and also indirectly affect nutrient availability (nutrients releasing from nonliving organic matter decomposition by soil microorganism activity and mineral nutrients moving from local toward plant root) for plant growth. Most studies conducted over the past 20 years on the effects of elevated CO₂ on plant biochemical composition have been conducted with amply watered plants growing in pots or chambers where water was unlikely to be limiting. Far fewer studies have examined the interacting effects of water supply and elevated CO₂ on plant metabolite levels. It was reported that although water-stress constrains the increase in growth exhibited under optimal soil moisture conditions and elevated CO₂, the effects of elevated
CO₂ on metabolite levels and plant water-use efficiency (WUE) tend to be greatest when plants were grown under conditions of water stress (Lavola and Julkunentiitto, 1994; Lawler et al., 1997). The potential for water stress to modify plant responses to elevated CO₂ is particularly relevant for predicting effects on plant quality in natural plant communities where competition for limiting water resources is often a key factor. Therefore, studying effects of elevated CO₂ on plant quality is critically dependent on experimental design, particularly, ample water versus water stress treatments.

In this study, sorghum samples were collected from a Free-Air CO₂ Enrichment (FACE) experiment designed with adequate and deficient water regimes at Maricopa, Arizona. The concentrations of amino acids, uronic acids, hemicellulose, cellulose, lignin, phenolics, C & N content, and C/N ratio in leaves, stems and roots of sorghum residues were analyzed. The objectives of our research were (1) to determine the major biochemistry of senesced leaves, stems and roots of sorghum grown under elevated CO₂, and (2) to address the interactive effects of CO₂ and H₂O on sorghum chemical composition. We focused on the above chemical compounds because they are ubiquitous in terrestrial higher plants, because they have exhibited sensitivity to elevated CO₂, and also because they are critical to plant residue decomposition in the soil ecosystems, and thus will influence the fate of atmospheric C sequestrated by terrestrial ecosystems.

Materials and Methods

FACE design

The C₄-sorghum FACE experiment was conducted at the University of Arizona Maricopa Agricultural Farm during the 1998 and 1999 growing seasons, with plant
samples for this study collected at the end of 1999 growth season. The FACE site is located 50 km south of Phoenix, Arizona (33.1°N, 112.0°W). The climate zone in this area is a typically desert regime, with a mean annual temperature of 21.8 °C and a maximum of 33.5 °C for the month of July recorded by the National Weather Service at Sky Harbor. The total annual precipitation is about 150 mm. Because of high temperature, low summer precipitation and southwest exposure, most agriculture can not be sustained without irrigation. The FACE experiment was set on alluvial plain and soil on this site is classified as a Trix clay loam (fine-loamy, mixed calcareous, hyperthmic typic Torrifluvents (Post et al., 1988).

The FACE experiment contains 8 rings (25-m diameter) in a 2x4 factorial design, in which four were maintained at current ambient CO$_2$ level (“Control”: 360 µmol mol$^{-1}$), and four at elevated CO$_2$ (“FACE”: 560 µmol mol$^{-1}$). The location of replicates were randomly placed in a 12-ha sorghum field. Additional details of the FACE facility are provided by Ottman et al. (2001). Briefly, FACE plots were set to a target CO$_2$ level of 560 µmol mol$^{-1}$, a concentration likely to be reached by next century, as projected by IPCC (2001). Additional pure tank CO$_2$ was continuously applied in these plots though the PVC pipe-blower system from sorghum emergence to maturity. CO$_2$ concentration inside plots was automatically regulated by a computer system according to wind direction, wind speed, and CO$_2$ concentration at the center of each ring. An identical air blower system was also installed in the non-CO$_2$-enriched ambient Control plots to reduce the mirometerological error caused by air flow.

Each of the circular FACE and Control plots was also split in semicircular halves, with each half receiving either a well-adequate (Wet) or water-deficient (Dry) regime
using level-basin flood irrigation. Wet half-rings were supplied with adequate water (100% replacement of potential evapotranspiration (ET) when 30% of the available water in the soil was depleted). Dry half-rings were irrigated with about half as much water as the Wet treatment, and with only two irrigations per season. Total water applied was 1047 and 491 mm in 1999 for the wet and dry treatments, respectively.

Sorghum (*Sorghum bicolor* (L.) Möench) seeds were sown in north-south rows 0.76 m apart with 9.97 kg ha\(^{-1}\) (41 mm apart for 32 seeds m\(^{-2}\); plant density of 21 plants m\(^{-2}\)) on 14-15 June 1999. The first fertilizer was applied before emergence by air at a rate of 93 kg N ha\(^{-1}\) and 41 kg P ha\(^{-1}\). The second fertilizer was subsequently applied with 172 kg N ha\(^{-1}\) in irrigation to both the wet and dry plots to give a total of 265 kg N ha\(^{-1}\). Thus, the wet and dry treatments received the same level of P and N fertilization for the season, with the identical application of N and P fertilizer level to both FACE and Control plots as well.

*Plant sampling*

Sorghum plants were sampled at the end of growing season on November 17-18, 1999, when the sorghum was fully maturated, thereby representing the post-season plant residues input into the soil system. A total of 4 whole sorghum plants including seeds, leaves, stems and roots were randomly selected from each semicircular plot, dug from ground, and then brought to the laboratory where they were separated into roots, stems, leaves and seeds. Roots and shoots were separated from the first stem node that is just at ground level. Leaves were separated by cutting them from the stem at the collar (leaves are leaf blades and stems include leaf sheaths). Heads were removed from the top stem.
Because seeds are not returned to soil as plant residues, our analysis of sorghum biochemical composition did not include sorghum seeds. The partitioned sorghum tissues of roots, stems and leaves were pooled for each sampling plot, air-dried at room conditions, and ground through the 20-mesh sieve using a Millele grinder. The ground tissues were oven-dried for 48 hours at 60 °C before weighing for chemical analyses.

*Phytochemical analyses*

Analyses were carried out on duplicate samples from portions of oven-dried and ground materials described above. The molecular and element fractions of amino acids, uronic acids, hemicellulose, cellulose, lignin, phenolic compounds, carbon, nitrogen and C:N ratio were analyzed. Except for the lignin analysis that was conducted in Laboratory of Tree-Ring Research at the University of Arizona, all other chemical analyses were conducted at the USDA-ARS Southwest Watershed Research Center in Tucson, AZ. The biochemical fractionations were based on hydrolytic degradation of these organic compounds in the presence of different concentrations of sulfur acid. The specific chemical compounds were identified and quantified by high-performance anion-exchange chromatography (HPLC) with pulsed amperometric detection analysis (HPAEC-PAD) performed on a DIONEX DX-500 (Dionex Corp., Sunnyvale, CA) liquid chromatograph gradient pump module and a Model PAD2 detector, and a Hewlett-Packard 1800A GCD gas chromatograph equipped with a HP-Ultra 1 capillary column (25-m length, 0.2-mm column i.d., 0.33-um film thickness) and a mass selective (MS) detector. Digestion and hydrolytic degradation for each biochemical composition are briefly described here.
Amino acids were determined by digestion of the materials (20 mg) in screw-top test tubes with 2 ml of 4 M methanesulfonic acid, and the mixtures were autoclaved for 16 h at 121 °C (104 kPa) (Martens and Loeffelmann, 2003). Following digestion, the samples were titrated to pH 4-5 with 5 M KOH, centrifuged to remove precipitate, and then an aliquot of supernatant was diluted for analysis of amino acids and amino sugars on a Dionex DX-500 (Dionex Corp., Sunnyvale, CA) ion chromatograph equipped with a 25 µL injection loop and an AminoPac PA10 guard and analytical column (250 mm x 2 mm i.d.). Pulsed amperometric detection with a Dionex ED-40 electrochemical detector set in the intergrated pulsed mode with a disposable gold working electrode was used for assay. The AA standards [AA kit (catalog no. LAA-10) and standard solutions (catalog no.AA-S-18)] were obtained from Sigma Chemical Co., St. Louis, MO, and were used to map retention times and detector response.

Uronic acid content was determined enzymatically by the method described in Martens and Frankenberger (1990). To solubilize uronic acids, sorghum tissue samples (200 mg) in screw-top test tubes were treated with 0.3 ml of 6 M H₂SO₄ to which was added 6.9 ml of DI (deionized) water to result in 7.2 ml of 0.25 M H₂SO₄. The mixtures were then autoclaved for 30 min at 121 °C, and after cooling centrifuged to remove precipitate (rinsed with 1 ml DI water and centrifuged again). The supernatants were combined, titrated to pH 4-5 with NaOH and then diluted to 10 ml with DI water. A 1-ml aliquot was diluted to 5 ml with DI water, treated with the enzymes pectolyase (3 units ml⁻¹) and B-D-glucuronidase (30 units ml⁻¹) at pH 6.8 (phosphate buffer) and then the mixtures were incubated overnight at ambient temperatures (16 h). The enzyme-extract mixtures were passed through an activated strong anion (3-quanternary
proplylammonium, Cl⁻) exchange column and rinsed with 3ml water. The uronic acids were eluted with several milliliters of 0.1 M NaCl (pH 8.0) and quantified by ion chromatography with pulsed amperometric detection as discussed for amino acid analysis.

Hemicellulose carbohydrates were extracted at room temperature from 20 mg samples of sorghum residue materials in screw-top test tubes (15x125 mm) with 800 µl of 6 M H₂SO₄, shaken with mild vortex mixing for 30 min, diluted with 4.2 mL of DI water to 1 M H₂SO₄, and then autoclaved at 121 °C (104kPa) for 30 min (Martens and Loeffelmann, 2002). After digestion and cooling, the samples were centrifuged, washed with two aliquots of 1 ml DI water and centrifuged again between each rinse. The three supernatants were combined in 14 ml Falcon Sterile Tubes, titrated to pH 5.5-6.5 with 5N NaOH, and then diluted to 10 ml with DI water for the analysis of separated hemicellulose carbohydrate on a DX-500 ion chromatograph equipped with a CarboPac PA10 column.

Cellulose was analyzed using the residues after hemicellulose extraction. The wet residues were oven-dried at 60 °C overnight, 300 µl of 18 M H₂SO₄ was added to each sample, shaken and allowed to wet thoroughly for 30 min. After cellulose solublization in this strong acid, the samples were diluted to 1.5 M H₂SO₄ with an additional 3.6 ml of DI water, autoclaved at 121 °C (104kPa) for 30 min, and then centrifuged (2100 rpm, 20 min) to remove precipitates (rinsing once with 1 ml of DI water and centrifuging again). The supernatants were combined into 14 ml Falcon Sterile tubes, neutralized to pH5.5-6.5 with 5 N NaOH, and then diluted to 10 ml with DI water. Monosaccharides released
from sample cellulose solublization were identified and quantified on a Dionex DX-500 ion chromatograph.

Non-lignin phenolic acids of sorghum residue materials (50 mg) were extracted with 5 ml of 1 M NaOH for 16 h on a reciprocal shaker at ambient temperatures as described in detail by Martens (2002). Following extraction, the mixture was centrifuged (2300 R, 20 min), and rinsed with 1 ml water. The supernatants were heated at 90 °C for 2 h to release the conjugated phenolic acids. After the heated extract was cooled, the samples were titrated with 4 M HCl to pH < 2.0, diluted to 10 ml with DI water, and centrifuged to remove the precipitate. An aliquot (2-3 ml) of the supernatants was passed through a conditioned Varian (Varian Assoc., Harbor City, CA) Bon Elut PPL solid-phase extraction tube (the PPL tubes were conditioned by first passing 2 ml of ethyl acetate followed by 2 ml water) at ~5 ml min⁻¹ attached to a Visprep (Supelco, Bellefonte, PA). The tubes were then placed under a vacuum (~60 kPa) until the resin was thoroughly dried after which the phenolic acids (NLPs) were eluted with 1 ml of ethyl acetate into gas chromatography autosampler vials for quantification by a Schematizer 17A gas chromatograph equipped with a HP-Ultra 1 capillary column (25-m length, 0.2-mm column i.d., 0.33-um film thickness) and a mass selective (MS) detector.

For the lignin assays, residue samples (300 mg) were treated with 15 ml of 72% sulfuric acid at 20 °C for 2 hours, with frequent stirring. The mixture was then transferred to a 2000-ml flask and 560 ml of water was added to decrease the acid concentration to 3% (w/v). The hydrolysate was boiled for 4 hours, maintaining constant volume by covering the flask with 50-ml flask for condensation and frequently adding hot water. After boiling 4 hrs, the solution was allowed to cool down, and the insoluble
materials (lignin) settled. The lignin was filtered with a 30-ml filtering crucible mounted on a 2000-ml filtering flask equipped with a vacuum line, and then washed free of acid with hot water. The crucible with lignin was dried in an oven at 105±3 °C to constant weight and then the lignin was collected. The results were expressed as percentage on a total dry mass basis.

Statistical analyses

The differences between control and CO₂ enriched, and between dry and wet residues for biochemical composition were tested by paired-t test, with 4 replicates. For each residue sample, data were analyzed using one-way and two-way ANOVAs to test for significant effects of CO₂ and CO₂ x water interaction, respectively. A third factor in the ANOVA analysis was types of tissues. Three tissues (leaf, stem, and root) were tested for differences in chemistry with CO₂, respect to water, and their interaction.

Results

The biochemical composition of sorghum tissues was changed in response to CO₂ and water treatments, and the responses were variable and depended on compounds and tissues parts (Table.1).

Amino acids

Overall, FACE tended to reduce the amino acid content in sorghum tissues, but not consistently. Total amino acids decreased in FW leaf, FD stem and FD root by 26.3%, 31.6% and 15.0%, respectively (Table 1, 2). However, amino acids in FW root, FW stem
Table 1. Summary means of major biochemical composition of sorghum tissues derived from open-field experiment under FACE (elevated CO$_2$: 560 µmol mol$^{-1}$) and Control (ambient CO$_2$: 360µmol mol$^{-1}$) treatments. The values were calculated based on the replication, n=4.

<table>
<thead>
<tr>
<th>Chemical Compositions (mg g$^{-1}$)</th>
<th>Irrigation</th>
<th>Control</th>
<th>FACE</th>
<th>FACE/Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaf</td>
<td>Stem</td>
<td>Root</td>
<td>Leaf</td>
</tr>
<tr>
<td>Amino Acids</td>
<td>Dry</td>
<td>48.12</td>
<td>48.81</td>
<td>40.89</td>
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<tr>
<td></td>
<td>Wet</td>
<td>70.98</td>
<td>27.34</td>
<td>29.82</td>
</tr>
<tr>
<td></td>
<td>Dry:Wet</td>
<td>0.68</td>
<td>1.79</td>
<td>1.37</td>
</tr>
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<td>Uronic Acids</td>
<td>Dry</td>
<td>4.24</td>
<td>3.85</td>
<td>2.27</td>
</tr>
<tr>
<td></td>
<td>Wet</td>
<td>3.59</td>
<td>3.28</td>
<td>2.06</td>
</tr>
<tr>
<td></td>
<td>Dry:Wet</td>
<td>1.18</td>
<td>1.17</td>
<td>1.10</td>
</tr>
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<td>Hemicellulose</td>
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<td>213.00</td>
<td>257.82</td>
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<tr>
<td></td>
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<td>243.36</td>
<td>266.88</td>
<td>251.70</td>
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<td>0.97</td>
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<td>Cellulose</td>
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<td>39.18</td>
<td>117.00</td>
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<td>251.21</td>
<td>149.71</td>
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<td>Dry:Wet</td>
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<td>0.92</td>
<td>0.94</td>
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<td>Phenolics</td>
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<td>0.94</td>
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<td></td>
<td>Dry:Wet</td>
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<td>1.01</td>
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<td>59.19</td>
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<td></td>
<td>Dry:Wet</td>
<td>1.04</td>
<td>0.39</td>
<td>0.63</td>
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Table 2. Means (n=4) of amino acid concentration (mg g\(^{-1}\)) of sorghum tissues derived from FACE (560 µmol mol\(^{-1}\)) and Control (360 µmol mol\(^{-1}\)) CO\(_2\) treatments.

<table>
<thead>
<tr>
<th>Amino Acids (mg g(^{-1}))</th>
<th>Irrigation</th>
<th>Control</th>
<th>FACE</th>
<th>FACE/Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Leaf</td>
<td>Stem</td>
<td>Root</td>
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<td>16.14</td>
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<td></td>
<td>Wet</td>
<td>26.08</td>
<td>10.32</td>
<td>8.47</td>
</tr>
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<td>1.56</td>
<td>1.14</td>
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<td>lysine</td>
<td>Dry</td>
<td>2.54</td>
<td>2.52</td>
<td>2.19</td>
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<td>3.81</td>
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<td>1.86</td>
</tr>
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<td>0.67</td>
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<td>Dry</td>
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<td>1.21</td>
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<td>1.28</td>
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<tr>
<td>isoleucine</td>
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<td></td>
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<td>Dry:Wet</td>
<td>0.71</td>
<td>1.75</td>
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</tbody>
</table>

*—other amino acids include methionine, hydroxyproline, and histidine
and FD leaf showed either no difference or a slight increase (Table 1, 2). Viewed across all 20 amino acids tested (Table 2), the aspartic and ser/pro concentration in all parts of sorghum tissues decreased substantially in elevated CO$_2$ treatment under both dry and wet conditions. Regardless of water condition, average reductions of aspartic acids for leaf, stem and root were 8.0%, 42.7% and 33.1%, respectively, and ser/pro acids were reduced 5.1%, 54.0% and 14.0%, respectively. Arginine, a dominant parameter of the amino acids, decreased in all tissues under FD conditions and in FW leaves, but increased in FW stems and roots.Alanine, glycine, leucine, phenylalanine, glutamic acid, and tyrosine were increased markedly only in leaf under both FD and FW conditions, but not in stems and roots.

**Uronic Acids**

Effects of elevated CO$_2$ on sorghum uronic acids were strongly influenced by soil moisture status. Under water-stressed condition, elevated CO$_2$ increased sorghum uronic acids by an average of 10.0%, but reduced the uronic acid concentration by an average of 5.8% in sorghum tissues collected from the well-watered treatment (Table 3). Although leaves had higher uronic acid contents than stems and roots, stems and roots were more sensitive to the interaction of elevated CO$_2$ and water stress than leaves. Uronic acids were 11.4% and 2.8% lower in FW stems and roots than CW stems and roots, respectively, whereas FD stems and roots had 22.2% and 6.8% higher uronic acids than CD stems and roots, respectively. Among specific uronic acids, galacturonic was reduced by the elevated CO$_2$ and wet treatment, but it increased significantly under the elevated CO$_2$ and dry treatment by an average of 7.2% to 21.4%. Glucuronic acid showed no
Table 3. Means (n=4) of uronic acid concentrations (mg g\(^{-1}\)) of the sorghum tissues (leaf, stem and root) derived from FACE (560 \(\mu\)mol mol\(^{-1}\)) and Control (360 \(\mu\)mol mol\(^{-1}\)) CO\(_2\) treatments.

<table>
<thead>
<tr>
<th>Uronics (mg g(^{-1}))</th>
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<th>FACE</th>
<th>FACE/Control</th>
</tr>
</thead>
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<tr>
<td></td>
<td></td>
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<td>Root</td>
</tr>
<tr>
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<td>0.23</td>
<td>0.25</td>
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<tr>
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<td>Dry:Wet</td>
<td>1.90</td>
<td>2.70</td>
<td>1.55</td>
</tr>
<tr>
<td>Mannuronic</td>
<td>Dry</td>
<td>0.61</td>
<td>1.25</td>
<td>0.74</td>
</tr>
<tr>
<td></td>
<td>Wet</td>
<td>0.42</td>
<td>1.13</td>
<td>0.74</td>
</tr>
<tr>
<td></td>
<td>Dry:Wet</td>
<td>1.44</td>
<td>1.11</td>
<td>1.01</td>
</tr>
<tr>
<td>Galacturonic</td>
<td>Dry</td>
<td>2.33</td>
<td>1.78</td>
<td>0.46</td>
</tr>
<tr>
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<td>Wet</td>
<td>2.06</td>
<td>1.56</td>
<td>0.50</td>
</tr>
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<td>Dry:Wet</td>
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<td>1.14</td>
<td>0.93</td>
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<tr>
<td>Glucuronic</td>
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</tr>
<tr>
<td></td>
<td>Dry:Wet</td>
<td>1.09</td>
<td>1.11</td>
<td>1.24</td>
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<td>Total</td>
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<td>Dry:Wet</td>
<td>1.18</td>
<td>1.17</td>
<td>1.10</td>
</tr>
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</table>
significant difference among the treatments except FD stem increased 33.3%.

Mannuronic and guluronic acids were reduced by elevated CO₂ under either wet or dry treatments, especially in sorghum leaves.

**Hemicellulose Carbohydrates**

Overall, FACE reduced the average hemicellulose carbohydrate (HCC) concentration of sorghum tissues, except for FD leaf (Table 4). The depressed levels were varied among tissues parts and water treatments. Under ample water conditions, elevated CO₂ reduced HCC concentration of all sorghum tissues by 11.7% for leaves, 5.3% for stems, and 14.6% for roots. However, under dry conditions, elevated CO₂ only significantly reduced HCC concentration of roots by 19.5, whereas it increased HCC in FD leaves by 22.1% and had no effect on FD stem HCC.

Inspection of the individual constituents of HCC (arabinose, galactose, glucose, and xylose with trace levels of mannose), reduction of HCC was caused mainly by a decrease in dominant glucose, which comprised nearly half of HCC. Glucose was significantly reduced on average for all tissues by 6.4% to 42.1% under both FW and FD, whereas other major components of HCCs, such as arabinose, galactose, and xylose were significantly increased especially under FD conditions.

**Cellulose**

As expected, elevated CO₂ significantly increased sorghum CC content by 57.8% and 62.8% in wet and dry treatments, respectively, except for FW root in which cellulose was reduced by 10.9% (Table 4). Leaves had the highest cellulose rise of 168.6% and
Table 4. Means (n=4) of carbohydrate concentrations (mg g\textsuperscript{-1}) of the sorghum tissues (leaf, stem and root) derived from FACE (560 µmol mol\textsuperscript{-1}) and Control (360 µmol mol\textsuperscript{-1}) CO\textsubscript{2} treatments.

<table>
<thead>
<tr>
<th>Carbohydrates (mg g\textsuperscript{-1})</th>
<th>Irrigation</th>
<th>Leaf</th>
<th>Stem</th>
<th>Root</th>
<th>Leaf</th>
<th>Stem</th>
<th>Root</th>
<th>Face/Control</th>
</tr>
</thead>
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<td><strong>Hemicellulose:</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Rabinose</td>
<td>Dry</td>
<td>27.90</td>
<td>27.96</td>
<td>24.42</td>
<td>41.82</td>
<td>41.13</td>
<td>24.24</td>
<td>1.50</td>
</tr>
<tr>
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<td>Wet</td>
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<td>27.36</td>
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<tr>
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<td>1.02</td>
<td>1.16</td>
<td>1.21</td>
<td>1.13</td>
<td>1.38</td>
<td>1.04</td>
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<tr>
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<td>Dry</td>
<td>11.52</td>
<td>16.86</td>
<td>11.58</td>
<td>16.86</td>
<td>18.72</td>
<td>9.72</td>
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</tr>
<tr>
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<td>15.30</td>
<td>9.84</td>
<td>16.17</td>
<td>16.08</td>
<td>8.76</td>
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<td>0.74</td>
<td>1.10</td>
<td>1.18</td>
<td>1.04</td>
<td>1.16</td>
<td>1.11</td>
<td>1.05</td>
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<td>64.92</td>
<td>74.70</td>
<td>80.88</td>
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<tr>
<td></td>
<td>Wet</td>
<td>61.92</td>
<td>107.04</td>
<td>120.54</td>
<td>57.93</td>
<td>77.10</td>
<td>106.26</td>
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<tr>
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<td>1.19</td>
<td>1.01</td>
<td>1.16</td>
<td>1.12</td>
<td>0.97</td>
<td>0.76</td>
<td>1.16</td>
</tr>
<tr>
<td>4. Xylose</td>
<td>Dry</td>
<td>99.96</td>
<td>99.09</td>
<td>90.00</td>
<td>136.02</td>
<td>121.86</td>
<td>88.56</td>
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<tr>
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<td>Wet</td>
<td>131.94</td>
<td>111.33</td>
<td>73.62</td>
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<td>126.30</td>
<td>77.58</td>
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<tr>
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<td>0.89</td>
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<td>5.82</td>
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<td>4.32</td>
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<td>0.74</td>
<td>4.47</td>
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<td>5.85</td>
<td>26.64</td>
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<td>13.57</td>
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<td>4.25</td>
</tr>
<tr>
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<td>0.99</td>
<td>0.16</td>
<td>0.32</td>
<td>0.32</td>
<td>5.72</td>
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<td></td>
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<td>115.32</td>
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<td>0.97</td>
<td>0.95</td>
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<td>343.26</td>
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<td>359.28</td>
<td>369.81</td>
<td>278.7</td>
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<tr>
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<td>282.54</td>
<td>381.9</td>
<td>298.68</td>
<td>319.95</td>
<td>384.91</td>
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<td>0.90</td>
<td>1.04</td>
<td>1.12</td>
<td>0.96</td>
<td>1.08</td>
<td>0.86</td>
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</table>
106.9% under dry and wet conditions, respectively, as compared to the respective controls. The dominant glucose component of cellulose increased substantially as a result of CO$_2$, whereas other constituents had no clear pattern in response to elevated CO$_2$, suggesting that the increase in cellulose caused by CO$_2$ was largely attributable to the increase in glucose in cellulose structure.

**Lignin**

Although elevated CO$_2$ increased sorghum lignin content in both water-ample and water-deficient treatments (Table 5), only sorghum samples collected from dry treatments showed significant increase in lignin content as compared to the controls. On average, lignin content in whole sorghum tissues was 7.0% and 3.4%, respectively, higher in FD and FW treatments than their respective controls. Lignin concentration in roots was largely enhanced by elevated CO$_2$ compared with leaves and stems, with the greatest increase of 10.4% in FD root compared with CD root.

**Phenolics**

Elevated CO$_2$ in either well-watered or water-limited treatment significantly increased phenolic compound concentrations in roots by 18.1% (FW) and 39.7% (FD), whereas no significant difference was found in leaves and stems except for FD stems in which phenolic compound content was reduced by –36.0% (Table-5). In this study, we found that ferulic and cinnamic phenolic monomers were particularly abundant in sorghum tissues, but syngaldehyde, vanillin, acetovanillione, and acetosyringone were more sensitive to elevated CO$_2$, overall increasing in response to elevated CO$_2$ by 49.3%,
Table 5. Means (n=4) of non-lignin phenolic concentrations (µg g⁻¹) and lignin contents (µg g⁻¹) of the sorghum tissues (leaf, stem and root) derived from the FACE (560 µmol mol⁻¹) and Control (360 µmol mol⁻¹) CO₂ treatments.

<table>
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<th>Non-lignin Phenolic and Lignin</th>
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<th>FACE</th>
<th>FACE/Control</th>
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<td></td>
<td></td>
<td>Leaf</td>
<td>Stem</td>
<td>Root</td>
</tr>
<tr>
<td>1. 4OH-benzaldehyde</td>
<td>Dry</td>
<td>15.32</td>
<td>1.95</td>
<td>9.04</td>
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<td>Wet</td>
<td>3.36</td>
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<td>Dry:Wet</td>
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<td>0.70</td>
<td>1.00</td>
</tr>
<tr>
<td>2. Vanillin</td>
<td>Dry</td>
<td>26.68</td>
<td>12.33</td>
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</tr>
<tr>
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<td>Dry:Wet</td>
<td>1.40</td>
<td>1.09</td>
<td>1.66</td>
</tr>
<tr>
<td>3. p-Hydroxyacetophenone</td>
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<td>2.34</td>
<td>0.45</td>
<td>0.77</td>
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<tr>
<td></td>
<td>Wet</td>
<td>1.79</td>
<td>0.88</td>
<td>0.66</td>
</tr>
<tr>
<td></td>
<td>Dry:Wet</td>
<td>1.31</td>
<td>0.52</td>
<td>1.17</td>
</tr>
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<td>Wet</td>
<td>1.64</td>
<td>0.92</td>
<td>0.65</td>
</tr>
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<td></td>
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<td>1.03</td>
<td>1.58</td>
</tr>
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<td>14.96</td>
<td>10.35</td>
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</tr>
<tr>
<td></td>
<td>Wet</td>
<td>9.86</td>
<td>2.95</td>
<td>24.74</td>
</tr>
<tr>
<td></td>
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<td>0.55</td>
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<tr>
<td>6. p-hydroxybenzoic acid</td>
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<td>0.90</td>
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<td>7. Syngaldehyde</td>
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<td>0.42</td>
<td>0.99</td>
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## Table 5-continued

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<th>Wet 2</th>
<th>Wet 3</th>
<th>Dry:Wet 1</th>
<th>Dry:Wet 2</th>
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<td>8. Acetosyringone</td>
<td>Dry</td>
<td>13.73</td>
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<td>2.67</td>
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<td>Wet</td>
<td>14.89</td>
<td>6.21</td>
<td>3.47</td>
<td>13.17</td>
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<td>0.88</td>
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<td>Dry:Wet</td>
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<td>0.54</td>
<td>0.77</td>
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<td>0.46</td>
<td>1.95</td>
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<td>9. Cinnamic acid</td>
<td>Dry</td>
<td>144.67</td>
<td>442.26</td>
<td>611.44</td>
<td>171.32</td>
<td>249.19</td>
<td>918.82</td>
<td>1.18</td>
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<td></td>
<td>Wet</td>
<td>140.65</td>
<td>515.96</td>
<td>647.11</td>
<td>126.74</td>
<td>514.31</td>
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<td></td>
<td>Dry:Wet</td>
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<td>0.86</td>
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<td>0.48</td>
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<td>10. Syingic acid</td>
<td>Dry</td>
<td>36.48</td>
<td>73.56</td>
<td>102.54</td>
<td>42.31</td>
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<td>135.35</td>
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<td>Wet</td>
<td>28.22</td>
<td>72.73</td>
<td>131.56</td>
<td>20.43</td>
<td>60.22</td>
<td>117.87</td>
<td>0.72</td>
<td>0.83</td>
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<td>Dry:Wet</td>
<td>1.29</td>
<td>1.01</td>
<td>0.78</td>
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<td>0.97</td>
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<td>11. Ferulic acid</td>
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<td>318.96</td>
<td>395.52</td>
<td>311.54</td>
<td>338.45</td>
<td>253.45</td>
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<td>321.53</td>
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<td>Lignin (mg g⁻¹)</td>
<td>Dry</td>
<td>238.31</td>
<td>138.05</td>
<td>205.69</td>
<td>259.37</td>
<td>145.54</td>
<td>227.00</td>
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<td>219.48</td>
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27.8%, 16.0%, and 12.7%, respectively. Water stress enhanced the effect of CO₂ on sorghum phenolic contents.

C/N ratios

Regardless of water treatment, elevated CO₂ did not affect the C/N ratio of sorghum residues (Table 1). Although a significant increase in sorghum biomass was observed in this FACE experiment by Ottman et al. (2001), overall CO₂ treatment did not affect sorghum C concentration. We only found that carbon concentration of FACE roots was slightly increased by an average of 7.5%, but it was decreased in stems by average of 8.9% with no effect on leaves. Comparing CO₂ effects with water regime, water-stress enhanced CO₂ effects on stem and root C concentration. For example, C concentration was 7.2% lower in FW stem and 2.0% higher in FW root, whereas it was 10.6% lower in FD stem and 13.0% higher in FD root compared with their controls. Nitrogen concentrations averaged 8.3% higher in the leaf and stem derived from the FACE wet treatments. However, under dry conditions the effects of CO₂ on sorghum residue N content varied with different sorghum tissues: 11.3% higher in FD leaf, but 10.33% lower in FD root relative to CD leaf and root. Elevated CO₂ had no effect on stem N content in either the higher water or lower water treatment. Consequently, in contrast to prevailing findings for C₃ plants, elevated CO₂ did not cause a significant difference in the C/N ratio of sorghum tissues except in FD roots, for which enriched CO₂ increased the C/N ratio by 26.0%. However, compared with ample water treatment, water stress considerably reduced the C/N ratios of sorghum leaves, stems and roots by 0.5%, 61.9% and 26.4%, respectively.
Discussion

A few studies have investigated the variation in C$_3$ plant residue quality in response to elevated CO$_2$, but little information is available for C$_4$ species. We have shown significant variation in the biochemical composition of C$_4$-sorghum growing under elevated and ambient atmospheric [CO$_2$]. However, the magnitude and tendency of responses to CO$_2$ and water were generally dependent on sorghum tissues and chemical compounds. Consistent with the findings from C$_3$ plants, C-based structural compounds of C$_4$ sorghum including holocellulose and lignin increased in response to elevated atmospheric CO$_2$. Although HCC concentration was generally not different even lower was found in the roots collected from CO$_2$-elevated plot than from control plot. The total of HCC and CC increased in response to elevated CO$_2$. The magnitude of increase (60.3-5.8%) was similar to that observed in other studies with C$_4$ plants (Henning et al., 1996).

Lignin is a very important C-based structure compound. In our study, a more significant increase in lignin was found in roots than in leaves and stems treated with elevated CO$_2$ compared to ambient CO$_2$. This result agreed with the findings from C$_3$ cotton by Booker et al. (2000). All our data for carbon-based structural compounds were in apparent agreement with carbon source-sink hypothesis that predicts increased CBSSC concentration under elevated atmospheric CO$_2$ because of the nutrient limitation that promotes production of more carbon-based structural cellulosics and lignin from the extra non-structural carbohydrates. However, our FACE experiment was a managed ecosystem rich in fertilizer, which means that an increase in carbon-based structural materials caused by elevated CO$_2$ is not just limited to natural ecosystems with nutrient limitations, but it also occurs in agro-ecosystem with nutrient-rich conditions. It is difficult to
ascertain the specific cause of the increase in CBSSC under the fertilized agro-ecosystem. One likely explanation may be related to the definition of ‘nutrient limitation’, which may include other common elements and trace elements plant need in addition to nitrogen and phosphorus.

Non-lignin phenolics represent soluble carbon-based secondary metabolites that can be found in all terrestrial higher plants. In our study, phenolic content in FACE roots was significantly higher than that in control roots, although no significant difference was found in leaves and stems. It was reported that phenolic content in response to elevated CO$_2$ is associated with other plant chemical compound abundance, such as carbohydrates, amino acids, and N content. Bass (1989) considered that total nonstructural carbon in excess of those required for protein synthesis is the main factor affecting the increases of CBSC concentrations under elevated CO$_2$. On the other hand, Jones and Hartley (1999) invoked the presence of a common precursor between proteins and phenolics, the amino acid phenylalanine. The competition between protein and phenolics for limiting phenylalanine results in a trade-off between rates of protein versus phenolic synthesis and thus an inverse relation between protein and phenolic allocation. Our results agree with this hypothesis. In our study, higher phenolic content was accompanied by lower total amino acids extracted from protein, in FACE roots compared to control roots.

In this study, the C/N ratios of sorghum tissues appear not to be affected by CO$_2$. This is in contrast to findings of a number of previous C$_3$ plant studies (Nakano et al., 1997; Rogers et al., 1996). However, other research from agro-ecosystem studies indicates that elevated CO$_2$ does not significantly affect and may even reduce the C/N ratio of plants (Franck et al., 1997; Ghannoum and Conroy, 1998). The explanation of no
effect on C/N ratio could be either that the FACE is an N-rich agro-ecosystem or that C₄ plants have higher N-use efficiency (NUE) compared to C₃ plants (Brown, 1979; Pheloung and Brady, 1979). The higher NUE of C₄ plant results from a lower N investment in rubisco protein compared to C₃ plants, which reduces the N restriction of C₄ plants growing under elevated CO₂ conditions. Even so, our results imply that elevated atmospheric [CO₂] causes a shortage of N supply for the synthesis of plant protein, because of reduced transpiration rates at high [CO₂]. This, in turn, causes a lower flux of N through the soil to the root surface, thereby reducing N uptake (Conroy, 1992). The evidence for this hypothesis is seen in the synthesis of amino acids, namely proteins. A limitation of total amino acid synthesis of sorghum grown under elevated [CO₂] was observed in our study. Also, in terms of individual amino acid, decreased FW leaf amino acid content was largely caused by the decreased lower C/N content amino acids such as arginine (-47.7%), lysine (-19.0%), and cystine (-92.6%), which increased the C/N ratio of total amino acids in FW leaf by 14.1%.

Of particular interest in this study was the potential for sorghum residue chemical composition to be influenced by the interaction of CO₂ and H₂O. Although we did not address the effects of water on sorghum quality, our results show that water stress does tend to enhance the CO₂ effects on sorghum biochemical composition. For example, in the elevated CO₂ treatment, reduced soil water supply increases the concentrations of hemicellulose carbohydrates, amino acids, uronic acids and even nitrogen by 8.3%, 6.2%, 35.3% and 61.3%, respectively, at the whole plant level. Soil water stress is known to alter the plant physiological process and indirectly influence nutrient availability, which regulates synthesis of both first metabolites (e.g. carbohydrates) and typical secondary
metabolites (e.g. amino acid, phenolics, lignin, and cellulose). Under water-stress conditions, plants will utilize all the water that they can obtain, so that season-long cumulative water usage is minimally affected by elevated CO$_2$ (Wall et al., 2001). In fact, any reduction in water usage will reduce soil-water depletion, which may feed back and improve physiological processes such as photosynthesis, and thus the composition of plant biochemical compounds. In absolute terms, the greater benefit from long-term CO$_2$ enrichment occurs when water supply is nonlimiting, but the enhancement of relative change in biochemical compositions increases substantially when water is deficient. This is because the stomatal closing response combined with photosynthesis stimulation leads to increased water-use efficiency (WUE) at high CO$_2$ concentration.

Overall, our results indicate that elevated CO$_2$ does affect C$_4$-sorghum residue biochemical composition. Although some CO$_2$ effects were opposite to common findings with C$_3$ plants, (such as our finding of no significant effects on carbon content, nitrogen content, and C/N ratio), CO$_2$ effects on secondary materials were consistent with findings from C$_3$ plants (i.e. increases in cellulose, lignin and non-lignin phenolic contents, and decrease in amino acids. Our results also indicate that effects of elevated CO$_2$ on plant chemical composition is deeply modified by other environmental factors, evidence of which is that soil water stress enhanced CO$_2$ effects on sorghum biochemical composition. Because sorghum samples used in this research were collected at the end of growing season, CO$_2$-elicited changes in chemical composition of sorghum tissues will carry over to sorghum residue and litter as an input of new soil organic matter, and play a pivotal role in regulating the dynamics of sorghum litter decomposition and consequently, the fate of sorghum residue/litter C in soil ecosystem.
Acknowledgment

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APPENDIX C

Tissue Chemistry of Sorghum Grown under Elevated Atmospheric CO$_2$
Impacts SOC Decomposition Rate

Abstract

Substrate quality has been recognized as one of the most important factors regulating soil decomposition processes, and thus will influence the sequestration of carbon in soil ecosystems. Elevated CO$_2$ has been found to increase the cellulose, lignin and non-lignin phenolics, and decrease the amino acids of sorghum tissues derived from Arizona Maricopa Free Air-CO$_2$ Enrichment (FACE) experiment. In order to evaluate how the biochemical composition of sorghum tissues affects the decomposition rate, we conducted a 79-day laboratory incubation of soil (20g) amended with leaf, stem and root of sorghum tissues (50 mg each) that were collected from the FACE experiment in which sorghum was exposed to two concentrations of CO$_2$ (Control: 360 or FACE: 560 µmol mol$^{-1}$). Decomposition was quantified as microbially respired CO$_2$ and new soil organic carbon (SOC) incorporated into the soil after incubation. The cumulatively respired CO$_2$ –C from the soil amended with FACE sorghum tissues was on average 7.3% less than that from the soil amended with control sorghum tissues in the first 13 days of incubation, but thereafter the difference declined and became insignificant, especially for leaf and stem treatments. However, cumulative CO$_2$-C released from the soil amended with FACE roots remained 12.7% lower than the Control root treatment over the 79-d incubation. The lower decomposition of FACE tissues was also evidenced from $^{13}$C/$^{12}$C analysis of new organic C incorporated in soil, which showed on average 9.1 mg and 7.2 mg of new C incorporated into the soil amended with FACE and Control tissues, respectively, as determined by a carbon isotopic tracer ($\delta^{13}$C). FACE sorghum resulted in 26.4% more new C in the soil compared with Control tissues. We also observed loss of old SOC, but found no evidence indicating the decomposition of old SOC was stimulated by the
addition of the sorghum tissues. Statistical correlations found soil respired CO$_2$-C and new C input were related to phenolic acid concentration ($r^2 = 0.93$, $p = 0.002$), and lignin concentration ($r^2 = 0.90$, $p = 0.004$). C/N ratio was not detected to be significantly related to decomposition. Our results confirm slower decomposition of FACE plant tissue reported previously due to biochemical alteration of the plant tissue produced under elevated CO$_2$.

Keywords: Elevated CO$_2$; sorghum tissue biochemical composition; decomposition; carbon stable isotope

Abbreviation: FACE---Free-air carbon dioxide enrichment; SOC---soil organic carbon (%); CO$_2$-C---respired CO$_2$ carbon ($\mu$g); $C_{\text{new}}$ and $C_{\text{old}}$ ---carbon (%) derived from new sorghum residues and old native SOC, respectively. $C_{\text{total}}$---total SOC (%); $^{13}$C/$^{12}$C---the ratio of stable isotopic $^{13}$C to $^{12}$C.

Introduction

Rising atmospheric CO$_2$ level will not directly affect soil processes because of inherently high soil CO$_2$ concentration (Van Veen et al., 1991), but will indirectly affect soil microorganism activity and soil organic matter decomposition through the changes in the residue chemistry from plants grown under elevated CO$_2$ (O' Neill and Norby, 1996). Soil organic carbon (SOC) is the driving force of most microbially mediated processes because it constrains the supply of energy for biologic enzyme production and growth, and therefore, qualitative changes in plant composition affect both soil microbial community structure and their heterotrophic pathways (Hodge, 1996; Klironomos et al.,
These feed back to affect soil organic matter decomposition and soil organic matter synthesis through their specific heterotrophic pathways.

A number of qualitative factors control soil microorganism activity and plant residue decomposition rate. Among these decomposition factors, C/N ratio, N content, initial lignin content and lignin/N ratio have been found to be good predictors of plant residue degradability (Aber et al., 1990; Melillo et al., 1982; Taylor et al., 1989), but not in all the cases (Gorissen et al., 1995; Van Ginkel et al., 1996). Besides C-N-lignin factors, many other carbon-based secondary and structural compounds such as cellulose, hemicellulose, tannin, proteins, lipids and phenolic acids have been highly correlated with the decomposition rate of plant residue, but such studies are fairly rare, especially for CO₂-associated research. Freeman et al. (2001) demonstrated that phenolic compounds inhibit the biodegradation of organic materials. In a laboratory incubation experiment using different organic residues with varied chemical composition, Martens (2000) reported that plant residues containing higher carbohydrates and amino acids were degraded rapidly, whereas plant residues having high phenolic acid content resulted in more organic matter remaining in soil. However, studies correlating decomposition rate of plants grown under elevated CO₂ with systemic chemical compounds that were expected to be changed under elevated CO₂ have not been conducted. Elevated CO₂ has been reported to affect plant biochemical composition by increasing carbon-based secondary and structural compounds and decreasing N content and N-dependent biochemical compounds (Castells et al., 2002; Mooney et al., 1991; Peñuelas and Estiarte, 1998). Therefore, changes in decomposition rates of plant material derived from plants grown under elevated CO₂ have been predicted because of modification of litter
quality associated with high CO₂ levels (Lambers, 1993). However, these theoretical predictions of the decomposition rates of litter derived from plants grown under elevated CO₂ have very little supporting experimental evidence (Coûteaux et al., 1991).

Previous research on decomposition rates of plant residues formed under elevated CO₂ conditions is limited and the results from these studies have been contradictory. For example, King et al. (2001) found that decomposition rate of *Populus tremuloides*’ litter collected from open-top chambers under ambient and elevated CO₂ conditions did not differ. Others have observed decreased rates of decomposition of litter resulting from exposure of plants to elevated CO₂ (Ball and Drake, 1997; Cotrufo and Ineson, 1996). In a field experiment with litter bags, Sowerby et al. (2000) showed reported that *L. perenne* litter originally grown in elevated CO₂ consistently had a greater rate of decomposition than the litter grown in ambient CO₂. These variabilities may reflect differences in plant species response to CO₂, and the effects of environmental conditions under which plant residues were degraded. The decomposition rate of plant residues is regulated by a hierarchy of interacting physical, chemical and biotic factors (Couteaux et al., 1995; Heal et al., 1997). Response to elevated CO₂ may differ between plants with C₃ and C₄ photosynthetic pathways and even between plants of the same pathways, and their responses may be sensitive to other environmental factors (Norby and Cotrufo, 2001). However, under given environmental conditions, plant chemical composition as a microbial substrate is a major component controlling decomposition (O’ Neill and Norby, 1996).

Substrate chemical composition also determines whether or not the ‘priming effect’ occurs. Elevated CO₂ has been widely reported to increase biomass of plants when
they are exposed to high CO\(_2\). The prevailing concept is that newly added fresh plant materials are degraded rapidly because the acquisition of energy for microorganism activities from fresh organic carbon is greater than that available from the existing soil organic carbon substrate. The “priming effect” hypothesis states that the increase in fresh organic matter input to soil will increase soil microbial activity because of the higher availability of energy released from the decomposition of this fresh organic matter, followed by an overall increase in soil organic matter (SOM) decomposition and decrease in soil carbon storage (Maly et al., 1992). However, this hypothesis has not been proven experimentally (Fontaine et al., 2003). The hypothesis does not account for effects of the fresh organic carbon quality on soil microbial activity and the decomposition rate of organic carbon. It is commonly believed that low quality organic matter limits the amount of available energy for soil microorganisms, and in turn the rate of soil organic matter decomposition (mineralization). Whether or not higher inputs of organic matter will increase the content of soil organic matter (SOM) depends largely on organic material quality and the soil microbial decomposition pathways affected by the organic material quality.

The primary goals of this study were to (1) determine the decomposition rate of the sorghum residues developed under elevated and ambient [CO\(_2\)] in a Free-air CO\(_2\) Enrichment (FACE) experiment, (2) examine the relationship between biochemical compounds and the residue decomposition rates, and (3) assess whether there is a “priming effect” when adding the new organic matter to the soil. We hypothesized that the decomposition rate of FACE sorghum tissues would be reduced because of more recalcitrant carbon-based structural and secondary compounds produced in sorghum
grown under elevated CO\textsubscript{2}. We included leaf, stem and root of sorghum plants with different biochemical composition driven by elevated CO\textsubscript{2}, to assess change in decomposition rate of different plant compounds and tissues and to infer how these changes might translate into shifts in the decomposition of old soil organic matter.

**Materials and Methods**

*Soil and sorghum samples*

The soil and sorghum plant samples used in this research were collected from a Free-Air CO\textsubscript{2} Enrichment experiment conducted at the Maricopa Agricultural Center of University of Arizona, Arizona, USA. The detailed FACE methods have been given by Ottman et al. (2001) and Leavitt et al. (in revision). Briefly, the FACE experiment was continuously conducted during the 1998 and 1999 growing seasons to investigate the effects of elevated CO\textsubscript{2} on a C\textsubscript{4}-sorghum agroecosystem. It consisted of 8 rings, each 25 m in diameter, randomly distributed in a 12-ha of sorghum field. Of the 8 rings, four control rings exposed the sorghum crop to an ambient CO\textsubscript{2} concentration of 360 µmol mol\textsuperscript{-1} (Control treatment), while the other four FACE rings received elevated CO\textsubscript{2} with a target concentration of 560 µmol mol\textsuperscript{-1} (FACE treatment). Both Control and FACE rings were equipped with identical blower systems, but only the FACE rings received additional CO\textsubscript{2}, which was derived from wells in southwestern Colorado and somewhat enriched in \textsuperscript{13}C (\textsuperscript{13}C was -4.6‰ relative to the background air that averaged -9.4‰ in 1999). As a result, the \textsuperscript{13}C of CO\textsubscript{2} in the CO\textsubscript{2}-enriched rings averaged -7.6‰, higher than the \textsuperscript{13}C value (-9.4‰) of CO\textsubscript{2} in Control rings by 1.8‰, and consequently C\textsubscript{4}-
sorghum tissues had average $\delta^{13}$C values of -10.7‰ and -12.4‰, respectively, in FACE and Control treatments. For each ring, FACE and Control plots were also split into wet and dry sides in semicircular halves; the wet side received about twice the irrigation water of the dry site.

Soil samples used in this study were collected from the surface layer (0-15cm) of Control wet sites at the end of 1999 grow season. The soil is classified as a Trix clay loam: fine-loamy, mixed (calcareous), hyperthermic Typic Torrifluvents, (Kimball et al., 1992; Post et al., 1988). After collection, soils were transferred to the laboratory, air-dried at room temperature, sieved through a 2-mm sieve, and then mixed thoroughly to provide a uniform medium for the incubation experiments. This soil had the following physical-chemical properties: pH (water) =7.6; sand = 35%; silt =32%; clay= 34%; organic carbon = 0.69%; N=0.07%; $\delta^{13}$C = -21.75‰. Prior to sieving, visible stones and plant residues were removed.

Sorghum plants were collected from both elevated CO$_2$ (560 µmol mol$^{-1}$) and ambient CO$_2$ (360 µmol mol$^{-1}$) wet plots at the end of 1999 growing season. A total of 4 whole sorghum plants including leaf, stem and root were randomly dug from each plot and separated into leaf, stem and root. Roots and shoots were separated from the first stem node that is just at the ground level. Leaves were separated by cutting them off where the leaf meets the stem. The partitioned sorghum tissues of leaves, stems and roots were pooled for each treatment, air-dried at room temperature, and ground with Millele grinder (GE, Mexico) through a 20-mesh screen. The ground tissues were oven-dried for 48 hours at 60 °C before weighing for the decomposition experiment.
**Incubation procedures**

Homogenized soil (20g) was thoroughly mixed with 50 mg of one type of sorghum tissues (leaf, stem and root), which is equal to the addition of 6.5 tons fresh organic materials ha\(^{-1}\) soil. The mixtures of soil and sorghum tissues were placed into 250 ml flasks, which were then tightly covered with rubber stoppers through which two tubing outlets were made, one for respired CO\(_2\) air going out and the other for CO\(_2\)-free fresh air going in. The outlets were kept closed most of the time with clips. Prior to initiation of the experiment, the volume of each flask excluding the stopper was exactly measured by filling with a known volume of deionized (DI) water.

Duplicate flasks were set up for all sorghum leaf, stem, and root additions, together with 2 control flasks containing soil only, and incubated at 23-25 °C. The soil within each flask was kept at 95% of its water holding capacity through the experiment (79 days) by weekly addition of DI water to a pre-determined weight. The incubation was started by evenly adding 4 ml of DI water to each flask (-34 kPa), and flushing the unit with CO\(_2\)-free air.

** Decomposition rate measurement**

To determine soil organic matter decomposition rate, an infra-red gas analyzer (IR6A) connected to a computer system was employed to record the respired CO\(_2\) in the flask was flushed through the meter from the tubing outlet with CO\(_2\)-free air from the other tubing outlet. Flasks were thus aerated at the end of each measurement. The first measurement was conducted after 6 hours of incubation near the end of microorganism lag growth, and then measurements were conducted at 12-hour intervals during the first
week; thereafter measurements were reduced to once per day and once per week as the experiment progressed. Immediately after each measurement, the two tubing outlets in the stopper of the flask were closed again with clips and the last CO₂ concentration within the flask was recorded, from which the next measured CO₂ was subtracted for the calculation of net CO₂ produced during each incubation period since the last measurement. Rates of respired CO₂ production were calculated as the cumulative amount of CO₂-C with time. Net CO₂-C production attributable to the decomposition of new added organic matter at each measuring time was calculated by subtracting the mean value of the respired [CO₂] of 2 controls (soil alone) from each measurement of the sample (soil + tissues). At the end of the incubation experiment, the incubated soils were analyzed for organic carbon content and stable isotopic C composition.

New and old SOC determination

To separate new and old organic matter left in the incubated soil, we measured the carbon isotopic composition (δ¹³C/δ¹²C) of sorghum tissues and soil organic carbon before and after incubation. Because SOM in the FACE field site was mostly derived from C₃-plant (e.g., cotton, wheat) cultivated previously, the δ¹³C value of SOC was about -22.0‰, which was much lower than the δ¹³C value of C₄-sorghum tissues (-10.27‰ and -11.69‰ for FACE and Control tissues, respectively.). This difference in carbon isotopic composition of old and new C provided a sensitive C tracer and enabled us to follow the incorporation of newly added sorghum materials into the incubated soil.

Prior to measuring soil organic C isotopes (δ¹³C/δ¹²C) after incubation, inorganic C in the soil sample was removed by putting the wet soil sample (moistened to field
capacity with DI water) under strong HCl vapor in a desiccator container for 24 h as described in Harris et al. (2001). All the samples, including soil and sorghum tissues, were oven dried for 48 hrs at 60 °C before carbon content and isotopic ratio analyses. Carbon content and carbon isotopic ratio were determined on a continuous-flow isotope ratio mass spectrometer (Finnigan MAT Delta-Plus-XL). The carbon content was expressed in percentage, and isotopic ratio was reported as $\delta^{13}C_{\text{PDB}}$ in per mil (‰), according to the definition:

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

where R is the $^{13}C/^{12}C$ ratio of the sample or the standard (the international Pee Dee Belemnite: $^{13}C/^{12}C = 0.0112372$). The fraction ($f_{\text{new}}$) of soil C derived from added sorghum tissue was calculated as:

$$f_{\text{new}} = \frac{\delta^{13}C_s - \delta^{13}C_o}{\delta^{13}C_n - \delta^{13}C_o}$$

or, $f_{\text{old}} = 1 - f_{\text{new}}$

and $C_{\text{new}}\% = f_{\text{new}} \times C_{\text{total}}$ or $C_{\text{old}}\% = f_{\text{old}} \times C_{\text{total}}$

where $\delta^{13}C_s$, $\delta^{13}C_o$ and $\delta^{13}C_n$ represents the $\delta^{13}C$ values of soil sample, old SOC and new SOC, respectively. $f_{\text{new}}$ and $f_{\text{old}}$ are the fraction of new sorghum residues and old native soil carbon, respectively. $C_{\text{new}}$, and $C_{\text{old}}$ are the proportions (%) of new and old SOC. $C_{\text{total}}$ indicates total C of the soil sample. Here, “s” (soil sample) is the soil after incubation, “o” (old SOC) is the original SOC left in the incubated soil with no plant amendment, and “n” (new carbon) is the labeled sorghum carbon incorporated into the
soil. We used the δ^{13}C of sorghum tissues as the isotopic value of new soil organic carbon (δ^{13}C_n), and δ^{13}C of the soil organic carbon after incubation in the soil alone Control treatment as the isotopic composition of old soil organic carbon (δ^{13}C_o).

Statistics

Mean value and standard deviation (s.d.) of respired CO_{2}-C production, soil organic carbon contents before and after incubation, and plant and soil organic carbon isotopic composition were calculated for each treatment including the soils amended with high CO_{2} and ambient CO_{2} tissues and the unamended control soil alone. Differences of these variables between the treatments were tested by one-way ANOVA and paired-t test. Correlation and linear regressions were applied to cumulative CO_{2}-C and new input C against the tissue biochemical parameters. Regressions were tested across all tissues with n=12 (3 tissues × 2 CO_{2} × 2 duplication). Additionally, one-way ANOVA analysis was also performed to determine the significant differences of degradation between tissues of leaf, stem and root, and the differences of old and new soil organic C left in the incubated soil between treatments. Statistic software used in this study was MINITAB (Version 13, Silicon Graphics).

Results

Residue decomposition rate

Cumulative CO_{2}-C from the decomposition of sorghum tissues during incubation shows similar patterns for all tissues, with lower CO_{2}-C released from the soil amended with FACE tissues than with Control (ambient CO_{2}) tissues (Figure 1). However, the
Fig. 1 Mean accumulative CO$_2$-C (µg) released from incubated soil amended with FACE (F: 560 µmol mol$^{-1}$) and Control (C:360 µmol mol$^{-1}$) sorghum leaf (A), stem (B) and root (C) over 79-d incubation.
significance of the difference between respired CO$_2$-C productions varied with the tissues. The respired CO$_2$-C released from FACE leaf- and stem-amended soils were significantly (p < 0.05) different from ambient CO$_2$ leaf- and stem-amended soils only for the initial 13 days, and thereafter they were not significantly different. In contrast, CO$_2$-C produced from the decomposition of FACE roots was significantly (p<0.001) lower than that from the decomposition of ambient CO$_2$ root over the 79-d incubation.

Results of cumulatively respired CO$_2$-C over the 79-d incubation indicate the average decomposition of FACE tissues was significantly (p<0.05) slower (by 7.3%) than control tissues for the initial 13-d incubation (Table 1). For specific tissues, cumulative CO$_2$-C was lower by 3.2%, 5.9% and 12.7% for FACE leaf, stem and root, respectively. The peak reduction in soil respiration between FACE tissues and Control tissue treatment was observed for the initial 3-d incubation. Afterward, the differences in respiration rate declined and were not significant after 13 days of incubation, especially for leaf and stem treatments that showed similar values of cumulative CO$_2$-C after incubation for 79 days. For the root tissue, however, cumulative CO$_2$-C was on average 12.2% lower in the soil amended with FACE roots than Control roots over the whole period of incubation.

**Correlation between residue composition and decomposition**

The results of regression of newly incorporated C into the incubated soil and cumulative CO$_2$-C over the incubation against sorghum tissue chemical compositions show that correlation of chemical parameters with new incorporated C was better than with cumulative respired CO$_2$-C (Table 2). The total phenolic acids and galacturonic concentrations were significantly (p<0.05) correlated with both new input C
Table 1. Mean of cumulatively respired CO$_2$-C (µg) released from incubated soil with FACE ([CO$_2$]=560 µmol mol$^{-1}$) and Control ([CO$_2$]=360 µmol mol$^{-1}$) sorghum tissues, and the comparison to average cumulatively respired CO$_2$-C from FACE tissues incubated soil with the soil incubated with control sorghum tissues.

<table>
<thead>
<tr>
<th>Incubation Day</th>
<th>FACE tissue amended cumulative CO$_2$-C (µg)</th>
<th>Control tissue amended cumulative CO$_2$-C (µg)</th>
<th>Percentage Difference (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaf</td>
<td>Stem</td>
<td>Root</td>
</tr>
<tr>
<td>3</td>
<td>1024</td>
<td>1079</td>
<td>1487</td>
</tr>
<tr>
<td>13</td>
<td>2510</td>
<td>2372</td>
<td>2339</td>
</tr>
<tr>
<td>30</td>
<td>3362</td>
<td>3348</td>
<td>2920</td>
</tr>
<tr>
<td>79</td>
<td>4095</td>
<td>4295</td>
<td>3672</td>
</tr>
</tbody>
</table>

* significant level with T-test is p< 0.05; ns: not significant, p > 0.05.
Table 2. Linear regression (y=ax + b) of new soil organic carbon input after incubation and cumulative CO$_2$-C produced over incubation period against major sorghum tissue biochemical parameters (n=6).

<table>
<thead>
<tr>
<th>Chemical Parameter</th>
<th>Correlation with new input C</th>
<th>Correlation with cumulative CO$_2$-C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>Phenolic acids</td>
<td>247.9</td>
<td>-932.4</td>
</tr>
<tr>
<td>Syringic acid</td>
<td>26.3</td>
<td>-132.7</td>
</tr>
<tr>
<td>Acetovanillione</td>
<td>-0.24</td>
<td>2.87</td>
</tr>
<tr>
<td>p-hydroxybenzoic acid</td>
<td>10.6</td>
<td>-65.1</td>
</tr>
<tr>
<td>4OH benzaldehyde</td>
<td>2.1</td>
<td>-11.6</td>
</tr>
<tr>
<td>Vanillic acid</td>
<td>6.1</td>
<td>-35.2</td>
</tr>
<tr>
<td>Acetosyringone</td>
<td>-3.2</td>
<td>32.9</td>
</tr>
<tr>
<td>Cinnamic acid</td>
<td>174.6</td>
<td>-890.8</td>
</tr>
<tr>
<td>Lignin</td>
<td>4.9</td>
<td>-17.5</td>
</tr>
<tr>
<td>Uronic Acids</td>
<td>-0.5</td>
<td>6.8</td>
</tr>
<tr>
<td>Galacturonic acid</td>
<td>-0.6</td>
<td>5.4</td>
</tr>
<tr>
<td>Cellulose</td>
<td>8.2</td>
<td>28.6</td>
</tr>
<tr>
<td>Glucose</td>
<td>-35.3</td>
<td>370.3</td>
</tr>
<tr>
<td>Hemicellulose</td>
<td>8.3</td>
<td>195.7</td>
</tr>
<tr>
<td>Arabinose</td>
<td>-3.5</td>
<td>53.4</td>
</tr>
<tr>
<td>Xylose</td>
<td>-13.0</td>
<td>209.5</td>
</tr>
<tr>
<td>Amino acids</td>
<td>5.5</td>
<td>-15.3</td>
</tr>
<tr>
<td>C/N ratio</td>
<td>6.8</td>
<td>-9.2</td>
</tr>
</tbody>
</table>

The significant levels are considered as ‘*’---p<0.05; ‘**’---p<0.01; ‘ns’---p>0.05.
and cumulative respired CO$_2$-C, but higher $r^2$ values were found with new input C ($r^2 = 0.931$ and 0.929, respectively) than $r^2$ values with cumulative respired CO$_2$-C ($r^2 = 0.720$ and 0.846, respectively). The concentration of lignin was also found to have a high positive correlation ($r^2 = 0.898; p < 0.01$) with newly input C, but the correlation with cumulative respired CO$_2$-C over 79-day incubation was not significant. Similarly, the contents of arabinose in hemicellulose and glucose in cellulose structure were only significantly correlated with newly input C, not with cumulatively respired CO$_2$-C.

C/N ratio and amino acid concentrations were not significantly correlated with either newly input C or cumulative respired CO$_2$-C, although C/N ratio had a high positive relationship ($r^2 = 0.452$) with newly input C. For other biochemical compounds such as cellulose, uronic acids and other monosaccharides, all the regressions, despite the high $r^2$ values for some chemical compounds, were not statistically significant because of the low number of cases.

**New and old SOC estimation**

Newly input and old soil organic carbon left in the incubated soil after 79 days of incubation were estimated with carbon isotopic mixing models, using carbon isotopic composition ($\delta^{13}C$) of sorghum tissues and SOC before and after incubation (Table 3). The $\delta^{13}C$ values of sorghum plant and SOC after incubation show sorghum tissues grown under elevated CO$_2$ were significantly (T-test $p <0.001$) more $^{13}C$ enriched (average $\delta^{13}C$ value of -10.3‰) than those grown under ambient CO$_2$ (average $\delta^{13}C$ value of -11.7‰) (Figure 2). The shift in $\delta^{13}C$ was approximately the same for all types of tissues ($\Delta = 1.4\%$), indicating that the isotopic composition of the assimilated CO$_2$ was constant.
Fig. 2 Mean carbon isotopic compositions ($\delta^{13}C$) of soil SOC after incubation (A) and sorghum tissues (B) collected from FACE (elevated [CO$_2$]= 560 µmol mol$^{-1}$) and Control (ambient [CO$_2$]=360 µmol mol$^{-1}$) plots. Vertical bars indicate standard deviation (n=2).
Table 3  Means ± standard deviation of resulting δ^{13}C values and soil organic carbon (SOC) content before and after incubation of 20g soil amended with 50 mg of sorghum tissues derived from elevated (560 µmol mol^{-1}) and ambient (360 µmol mol^{-1}) CO_{2} treatments in Maricopa FACE experiment, including new and old SOC calculated with isotopic mixing model. Also shown are the differences of isotopic composition and decomposition of sorghum grown under elevated CO_{2} and ambient CO_{2} conditions.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Elevated CO_{2}</th>
<th>Ambient CO_{2}</th>
<th>Elevated CO_{2} increase(+) or decrease(-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sorghum tissue δ^{13}C_{n} (%)</td>
<td>-10.27±0.16</td>
<td>-11.69±0.14</td>
<td>+1.42*</td>
</tr>
<tr>
<td>SOC δ^{13}C_{s} after incubation (%)</td>
<td>-20.56±0.17</td>
<td>-20.79±0.09</td>
<td>+0.23*</td>
</tr>
<tr>
<td>Control (soil alone) SOC δ^{13}C_{o} (%)</td>
<td>-21.33±0.07</td>
<td>-21.33±0.07</td>
<td>N/A</td>
</tr>
<tr>
<td>New C input fraction (f_{new})</td>
<td>0.070±0.010</td>
<td>0.046±0.009</td>
<td>+0.024*</td>
</tr>
<tr>
<td>Old C fraction (f_{old})</td>
<td>0.930±0.010</td>
<td>0.944±0.009</td>
<td>-0.014_{ns}</td>
</tr>
<tr>
<td>Original SOC before incubation (C%)</td>
<td>0.69</td>
<td>0.69</td>
<td>N/A</td>
</tr>
<tr>
<td>Original SOC before incubation (mg C)</td>
<td>138</td>
<td>138</td>
<td>N/A</td>
</tr>
<tr>
<td>SOC after incubation (C%)</td>
<td>0.65±0.034</td>
<td>0.64±0.031</td>
<td>+1.56_{ns}</td>
</tr>
<tr>
<td>Control SOC after incubation (C%)</td>
<td>0.583±0.01</td>
<td>0.583±0.01</td>
<td>N/A</td>
</tr>
<tr>
<td>New C loss during incubation (C%)</td>
<td>58.05±6.43</td>
<td>67.34±5.12</td>
<td>-9.29_{ns}</td>
</tr>
<tr>
<td>Old C loss during incubation(C%)</td>
<td>11.93±3.70</td>
<td>11.72±1.75</td>
<td>+0.21_{ns}</td>
</tr>
<tr>
<td>New C input (mg C)</td>
<td>9.12±1.59</td>
<td>7.18±1.13</td>
<td>+27.02_{ns}</td>
</tr>
<tr>
<td>Old SOC remains (mg C)</td>
<td>121.54±5.59</td>
<td>121.82±5.21</td>
<td>-0.23_{ns}</td>
</tr>
</tbody>
</table>

* Significant level with T test is p<0.05; ns: not significant, p> 0.05.
over the FACE growing season. The $\delta^{13}C$ of incubated SOC reflects differences in FACE sorghum tissues compared with control tissues (-20.6‰ and -20.8‰ for FACE and Control tissue amended soils, respectively). However, the differences in $\delta^{13}C$ values of SOC were much smaller than those of sorghum tissues because of involvement of bulk SOC. Compared with the $\delta^{13}C$ (-21.3‰) of the incubated soil control, the shift of $\delta^{13}C$ values of SOC amended with either FACE or Control sorghum tissues suggests that new sorghum carbon was incorporated into the soil carbon pool.

With the incorporation of new organic carbon, the total SOC content of incubated soil with the addition of sorghum tissues should increase. However, the total SOC contents of the soil after incubation, 0.65% and 0.64%, respectively, for FACE and Control tissue amended soils, were lower than the carbon content (0.69%) of the soil before incubation, indicating at least some old SOC must have been lost as respired CO$_2$ during the incubation. Based on the calculation with the isotopic mixing model, new carbon fractions ($f_{new}$) in SOC after incubation were on average 7.0% for the soil amended with FACE sorghum tissues and 4.6% for the soil amended with ambient CO$_2$ tissues. About 9.12 and 7.18 mg of new carbon, respectively, were incorporated into the 20g of soil amended with FACE and Control sorghum tissues (i.e., 0.27% increase in soil carbon after FACE sorghum amendments ($p<0.01$)). New organic carbon inputs were increased by 25.5%, 25.9% and 29.3% for the soils amended with leaf, stem and root, respectively. In other words, the initially added FACE sorghum tissue carbon was lost slower than control sorghum tissue. The initial added carbon lost was 63.9%, 59.0% and 51.2% for FACE leaf, stem and root, respectively, and 71.0%, 69.6% and 61.5% for control leaf, stem and root, respectively, with an average of 58.0% and 67.3% loss for FACE and
Control sorghum tissues, respectively. These results are consistent with what we observed from the measurement of respired CO$_2$ production, with a lower degradation of FACE tissues than ambient CO$_2$ tissues. It should be pointed out that the difference of the cumulative CO$_2$-C loss was much less than loss of added carbon calculated with isotopic mixing model between FACE and Control tissues amended soils. We suspect that the difference of cumulative CO$_2$-C released from soils amended with FACE and Control tissues are under-estimated because of neglect of the C losses during the process of measuring carbon and isotopic composition after incubation, and also because of involvement of old bulk soil organic C decomposition in cumulative CO$_2$-C.

Original soil organic carbon decreased by 15.8%, 8.5% and 11.5% for the soil amended with FACE leaf, stem and root, respectively, and by 10.3%, 13.7% and 11.18% for the soil amended with Control leaf, stem and root, respectively, with an average of 11.9% for FACE tissue amended soil and 11.7% for Control tissue amended soil (Fig.3). However, this difference in original SOC decomposition between addition of FACE tissues and Control tissues was not statistically significant. Additionally, original SOC remained at 0.61% and 0.62% after incubation, for the soil with addition of FACE tissues and Control tissues, respectively (no statistical difference). Even if compared with the control incubation treatment (soil alone), for which 0.58% of the original SOC remained after incubation, there was no significant difference in original SOC decomposition among treatments.
Fig. 3 Mean new organic C (%) (A) and old soil organic C (%) (B) in the soil after 79-d incubation with FACE (elevated [CO$_2$]= 560 µmol mol$^{-1}$) and Control (ambient [CO$_2$]=360 µmol mol$^{-1}$) fumigated sorghum leaf, stem and root. Vertical bars indicate standard deviation (n=2)
Discussion and Conclusions

We investigated the effects of tissue quality of plants grown under elevated atmospheric \([\text{CO}_2]\) on decomposition rate by using laboratory incubation of soil amended with sorghum tissue derived from Maricopa FACE experiment. We found that the sorghum tissues developed under elevated \(\text{CO}_2\) were degraded slower than ambient \(\text{CO}_2\) sorghum. Analysis of newly input C showed that 27.0% more new carbon was input to the soil amended with sorghum tissues derived from elevated \(\text{CO}_2\) versus ambient \(\text{CO}_2\). Our data agree with earlier studies that investigated the effects of elevated \(\text{CO}_2\) on plant quality and plant decomposition. For example, Gorissien, et al. (1995) found in a 64-d laboratory experiment that the decomposition of \(^{14}\text{C}\) labeled grass roots grown under an elevated atmospheric \(\text{CO}_2\) treatment decreased by 24% compared with grass roots grown in ambient air. Using cotton leaf, stem and root derived from a pot experiment, Booker et al. (2000) reported that cumulative \(\text{CO}_2\) released was lower in soils amended with cotton residues from the elevated \(\text{CO}_2\) (722 \(\mu\text{mol mol}^{-1}\)) than control \(\text{CO}_2\) (390 \(\mu\text{mol mol}^{-1}\)) treatment, with a 10% reduction of cumulative respiration from the soils amended with high \(\text{CO}_2\)-treated leaf residue during the first 16 d of incubation and 10 to 14% lower respiration in the treatment with high \(\text{CO}_2\)-treated stems or roots during the entire duration of the experiment. In microcosm experiments, Coûteaux et al. (1991), Cotrufo et al. (1994), and van Ginkel et al. (1996) also found a significant decrease (17% to 28%) in decomposition rates of naturally senescent sweet chestnut leaf litter, birch and spruce leaves and \textit{Lolium perenne}\ L. roots following growth in approximately twice-ambient \(\text{CO}_2\). The decomposition rate of sorghum residues derived from open-top field chambers was studied by Torbert et al. (1998) who reported that cumulative respiration after
incubation for 60 days decreased 19.8% in soil amended with sorghum residues that had been treated with twice-ambient CO₂ compared with ambient CO₂ treated. In agreement with other researchers, we believe that the lower decomposition ratio of FACE to ambient CO₂ fumigated sorghum tissues in the present study is likely related to change in the chemical quality of the tissue material because the incubation experiments were conducted under identical environment conditions.

Many researchers have suggested that reduced N content and increased C/N ratio of plants grown under elevated CO₂ condition are two important factors restricting plant residue decomposition. In our study, however, we found chemical characteristics, other than N content and C/N ratio, were important in controlling the decomposition of sorghum tissues. Regression of sorghum tissue degradation against chemical composition showed that C/N ratio was not significantly related to the decomposition, whereas phenolics and lignin were major factors in determining sorghum tissue decomposition rates. Notably, phenolic concentration was significantly related to both newly input C and cumulatively respired CO₂-C, and explained most of the variation in decomposition rates. On the other hand, the larger differences of phenolic acid content coincidental with larger difference of decomposition rate were found from root materials, suggesting that phenolics exerted a greater control over root tissue decomposition (Martens, 2002). This effect could be attributed to the difference in microbial metabolic functions (Kuiters, 1990), interaction between tissue phenolic content and other chemical compounds (Vaughan and Ord, 1991), and toxicity of the phenolics to soil fungal and enzyme activities (Dixon and Paiva, 1995), but our study was not intended to investigate these mechanisms.
The absence of a significant correlation between C/N ratios with either newly input C or cumulatively respired C in the present study may be a result of the fact that the C/N ratio of the tissue was not affected by the elevated CO$_2$ in the nitrogen-fertilizer-rich FACE agroecosystem. In a recent review, effects of elevated CO$_2$ on the C/N ratio of plant residues and relationships of C/N ratio with the residue decomposition rates have been questioned. Cotrufo and Ineson (1995) suggested that increasing C/N ratio of plant residues induced by elevated CO$_2$ treatment occurred only in the unfertilized soil, and noted that higher decomposition rate of birch residues derived from elevated CO$_2$ was mainly attributed to litter quality factors other than C/N ratio. In a field experiment, Van Ginkel et al. (1996) found reduction in the decomposition rates of ryegrass root tissues generated under elevated CO$_2$, but they had same C/N ratio. After investigating the elevated CO$_2$ effect on the quality and decomposition of C$_3$ and C$_4$ plants, Sowerby et al. (2000) pointed out that although elevated CO$_2$ resulted in a higher C/N ratio of both C$_3$ (Yorkshire: H. lanatus) and C$_4$ (Kikuyu: P. clandestirum) litter, the C/N ratio was a poor indicator of the subsequent decomposition rate of the litters. Nevertheless, in agreement with Cotrufo and Ineson (1996), elevated CO$_2$ reduces decomposition rate of plant residues only when significant changes in substrate quality occur.

In the present study, the impact of tissue chemical composition on decomposition rate in soil is also demonstrated from the analysis of newly input C and old C remaining using the isotopic tracer method. The results showed that new C input to soil amended with FACE sorghum tissues was higher than the soil amended with Control sorghum tissues, and the difference was statistically significant, confirming sorghum plant tissue grown under elevated CO$_2$ was degraded slowly. Old soil organic carbon was also lost,
but the difference was not statistically significant. These results do not support the ‘priming effect’ hypothesis, and they contrast with the findings of Maly et al. (1992). From laboratory microcosm studies on C and N cycling during the decomposition of organic substrates in soil, Maly et al. (1992) reported that a positive ‘priming effect’ was induced by the substrate, with the effect proportional to the amount of substrate added. In contrast, our data did not show any stimulation of the decomposition of the original soil organic matter induced by the addition of sorghum tissues, for any of the different types of tissue (i.e., no apparent ‘priming effect’ was observed). Statistical analysis between either unamended soils and amended soils (plant tissue added), or FACE tissues and ambient grown tissue amended soils did not provide any evidence to support the ‘priming effect’. Among all treatments there was no significant difference in old soil organic carbon, but there is in newly input carbon.

Our results must be tempered by the realization that although the residues were produced under open-field ecosystem conditions, closely similar to natural systems, the laboratory incubations were far removed from realities of residue decomposing in the field (the lack of a complex food web, soil microbial interaction, etc.). However, significant reductions in the decomposition rates of plants grown under elevated CO₂ have been observed as well in natural field conditions (Cotrufo et al., 1998; Van Ginkel et al., 1996). Furthermore, the results presented here, using sorghum tissues derived from the Arizona FACE experiment, confirm the results obtained from the FACE field study by Leavitt et al. (in revision), who demonstrate that the inputs of new SOC in the FACE plots were 5.8% and 7.7% higher than in Control plots for wet and dry treatment, respectively, over two year experiment even though the increment was lower than that we
detected in the laboratory incubation study. Regardless of the difference, the evidence suggests the rate of microbial metabolism in decomposing C₄-plant residues may be reduced in a future, high-CO₂ world. The reduction is mainly attributed to the change in residue biochemical composition when the plants are exposed to an elevated-CO₂ atmosphere.

Although under elevated CO₂ conditions, net primary production of C₄ plants is not significantly increased (+8% and +3% increase in aboveground sorghum biomass under dry and wet condition, respectively, in this sorghum FACE experiment (Ottman et al., 2001)), changes in biochemical composition, especially increasing phenolics and lignin, may be accompanied by a slow-down of decomposition rates, and will result in a net increment of C-storage in the soils of C₄-plant ecosystems. C₄ plants occupy about 20% of terrestrial land area and may, therefore, represent a potential increasing sink for the ‘missing (excess) carbon’ identified from the recent estimates of the global carbon cycle (Lal, 2004). Even small reductions in the decomposition rate of C₄ plant residues may result in significant mitigation of harmful elevated atmospheric CO₂. Another major remaining question regards which soil carbon pools will acquire the increased new C, which will determine the long-term effect of elevated CO₂ on soil carbon storage and the stabilization of newly input carbon. This area needs to be further study.
Acknowledgments

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References


APPENDIX D

Dynamics of Labile and Recalcitrant Soil Carbon Pools in a Sorghum Free-Air CO$_2$ Enrichment (FACE) Agroecosystem

Abstract

Knowledge of the effects of elevated CO$_2$ on dynamics of soil carbon pools can better define the ability of terrestrial ecosystems to sequester global carbon. In the present study, 6 N HCl hydrolysis and stable-carbon isotopic ($\delta^{13}$C) analysis were used to investigate the 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, with elevated CO$_2$ (FACE: 560 µmol mol$^{-1}$) and ambient CO$_2$ (Control: 360 µmol mol$^{-1}$) and well-watered (wet) and water-stressed (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 soil organic carbon into the slow-decay carbon pool. Also, isotopic mixing models reveal 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. Mean residence time (MRT) of bulk soil carbon at the depth of 0-30 cm increased from 14-22 years in the Control plots to 15-58 years in FACE plots. 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 that in total SOC. Our results imply that terrestrial agroecosystems may potentially play a critical role in mitigating excess CO$_2$ in the future atmosphere.
Key words: Elevated CO₂; carbon stable isotopes; soil recalcitrant and labile carbon pool; dynamics of soil organic carbon; soil organic C mean residence time; sorghum FACE

Abbreviation: FACE---free-air CO₂ enrichment; SOC---soil organic carbon; Cₜ---total carbon; Cᵣ---recalcitrant carbon; Cᵱ---labile carbon; CD---control (ambient CO₂: 360 µmol mol⁻¹) dry (water-stressed); CW---control (ambient CO₂) wet (well-watered); FD---FACE (560 µmol mol⁻¹) dry; FW---FACE wet; MRT---mean residence time; Xₜ---the original SOC remaining after year t; X₀---the SOC content prior to CO₂ experiment; k---the decomposition constant; t --- the time elapsed since CO₂ fumigation

Introduction

Elevated CO₂ 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 CO₂ and because of the short-term nature of most CO₂-enrichment experiments. Soil is the biggest carbon pool in the Earth’s terrestrial ecosystem, containing 3000-5000 Pg C in organic matter, which is about 6 and 7 times as large as the C content in the atmosphere and land biosphere, respectively (Lal, 2004; Lal et al., 1997; Stevenson and Cole, 1999). Given the size of the soil C pool, a very slight increase in soil carbon content could cause meaningful sequestration of excess atmospheric CO₂. However, detecting the change in total SOC content is very difficult simply because soil is a large C reservoir and the changes of parameters in question are small, especially for short-term experiments (Hungate et al., 1996). Presently, total soil C content is
determined with an accuracy of ±2% of the total amount present, which means that management effects cannot usually be determined until after decades (Paul et al., 2001b). This is why the behavior of soils as C sinks or sources under increased atmospheric CO₂ has been poorly understood.

Determination of CO₂ effects on soil carbon processes requires 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, soil organic carbon components can be fractionated into several different carbon pools with various MRTs. Each carbon pool plays a very different role in soil organic carbon dynamics and soil carbon sequestration. Undoubtedly, if a soil system encourages the translocation of soil organic matter into labile pools with short-MRT under elevated CO₂, commonly associated high microbial activity results in rapid decomposition and decrease in soil carbon storage. In contrast, if elevated CO₂ 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, the division of SOC into different kinetic pools is essential for understanding the dynamics of the SOC under a given environmental condition such as elevated CO₂ (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; Jastrow et al., 1996; Trumbore, 1993), humic fractionation (Campbell et al., 1967; Nissenbaum and Schallinger, 1974),
molecular fractionation (Johnson, 1986; Martens and Frankenberger, 1991; Sorensen and Paul, 1971), and conceptual fractionation with models (Hsieh, 1989; Parton et al., 1987). Unfortunately, none of those fractionation methods can satisfactorily and practically separate a set of soil carbon pools on a stability basis. For example, particle size and density fractionations have been shown to yield pools of SOC with different properties regarding chemical composition and turnover (Christensen, 2001; Roscoe et al., 2001; Tiessen and Stewart, 1983). The MRTs associated with the particle sizes, however, had little in common with real correlations between distributions of particle size and SOC decomposition rate in the field soil profile (Balesdent, 1996; Christensen, 1992; Jastrow et al., 1996; Roscoe et al., 2001). One of the traditional methods separated total SOC into humic fractions. Although humic acid and humin fractions were usually older (1130-1410 yrs) than the corresponding fulvic acid fraction (50-550 yrs), Campbell et al. (1967) found that a portion of humic acid and humin substances were still active with a short MRT (25-465 yrs), the same as fulvic acid, indicating incomplete separation of labile from recalcitrant pool. Modeling fractionation usually separated SOC into 2 to 5 pools. For example, (Parton et al., 1987) simulated SOC levels in Great Plains grasslands using a model to separate soil carbon pools with various MRTs into a metabolic pool (0.1-1yr), 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 understandable analytical information on the physical and chemical properties of the SOC. Molecular fractionation, aided by highly specialized equipment, 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 MRT of years to a few decades, which is affected by variations of environmental factors over short periods, and a recalcitrant pool with MRT 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 the effects of elevated CO$_2$. In the present study, we used 6 N HCl hydrolysis (Campbell et al., 1967; Leavitt et al., 1996) to separate SOC into two pools, taking the residue fraction after the hydrolysis as a recalcitrant pool and the hydrolysate (or supernatant) fraction as a labile pool.

The fractionation with 6N 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 hydrolysates after 6N 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), indicating that the hydrolysable organic carbon fraction is more active and non-hydrolyzable 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 $170 \pm 60$ BP to $4900 \pm 50$ BP, were much older than the carbon in the hydrolyzate fractions in which
carbon age ranged from modern to $340 \pm 60$ 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 hydrolysable C fractions were generally several hundreds to thousands of years younger than the total SOC. (Collins et al., 2000), used 6N HCl hydrolysis, initially to divide US Corn Belt soil C into a resistant pool (residues of acid hydrolysis) and a hydrolysate pool. Then using a curve-fitting model of respired CO$_2$ released from laboratory incubation, they further divided the hydrolysate C into active and slow C pools. They reported that the active pool comprised 3 to 8% of the SOC with an average field MRT of 100d, and the slow pool comprised 50% -65% of SOC with MRTs of 12-28 yrs. Compared to the resistant pool, which constituted 56% of total SOC with a MRT of 2600 yrs, the active and slow pools could both be considered as modern. This implies that two labile pools and one recalcitrant pool provided necessary information on the long-term soil C dynamics response to the changes in environment such as rising CO$_2$ in the atmosphere.

It was reported that, besides old aromatic humics (Campbell et al., 1967), 6N HCl hydrolysis also leaves behind other resistant compounds derived from newly input plant materials in the residue, such as cellulose (Scharpenseel and Schiffmann, 1977), and lignin (Collins et al., 2000; Leavitt et al., 1996), waxes (Hobbie et al., 2002), and fat resins and suberins (Rovira and Vallejo, 2002). All of these compounds are highly resistant to chemical and biological degradation (Maier et al., 2000; Minderman, 1968). Such easily degraded compounds as amino acids, amino sugar, soluble carbohydrates and microbial biomass (Hobbie et al., 2002; Hu et al., 1997; Rovira and Vallejo, 2002) are
major components of the acid-hydrolyzable fraction. This further indicates that 6N HCl hydrolysis method effectively separates all labile carbon from the recalcitrant pool.

Carbon isotopic techniques using stable tracers ($^{13}$C/$^{12}$C) provide a highly accurate method to trace carbon transfer among the pools, which has been successfully used in studies of SOC dynamics (Leavitt et al., 1996; Leavitt et al., 1997; Leavitt et al., 1994; 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 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 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). The newly input sorghum residues were highly enriched in $^{13}$C compared to original SOC, which 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 (1) to separate soil labile and recalcitrant pools by using 6 N HCl hydrolysis, (2) to determine the transfer of new C$_4$-sorghum residues among these pools using isotope techniques, and (3) to 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. We assumed that the structure and size of the labile and recalcitrant carbon pools control the carbon cycle in the soil and thus play a vital role in the potential of soil carbon storage, and that knowledge of the dynamics of carbon content in labile and recalcitrant pools is especially important in the interpretation of soil carbon responses to elevated atmospheric CO$_2$.

Material and Methods

FACE site description

The sorghum [Sorghum bicolor (L.) Möench] Free-Air CO$_2$ Enrichment (FACE) experiment was conducted at the University of Arizona Maricopa Agricultural Farm (elevation: 358 meters, 33.1 °N, 112.0 °W), AZ, USA, continuously for two growing seasons of 1998 and 1999. The detailed methods have been given by Ottman et al. (2001) and Leavitt et al. (in revision). Briefly, the FACE experiment consisted of eight 25-m-diameter rings (2 CO$_2$ × 4 replicates), 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 from the date when 50% of the sorghum plants emerged until plant maturity, 24 hours per day. 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 µmol mol$^{-1}$ in FACE and Control plots, respectively. Average nighttime CO$_2$ concentrations were slightly higher at 603 and 607, and 428 and 433µmol mol$^{-1}$ at FACE
and Control plots, respectively. Each of 8 rings, for both FACE and Control, was also 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).

The soil in FACE site was classified as a Trix clay loam: fine-loamy mixed (calcareous), hyperthermic Typic Torrifluvents (Kimball et al., 1992; Post et al., 1988), 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 ranging from 30 to 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 a known volume of soil collected from FACE and Control replicate 1 and 2, and then oven-dried at 105 °C for more than 24 hours to constant weight). Soil organic matter originated from both native vegetations (e.g., CAM and C\(_4\) plants) and frequent cultivation of C\(_3\) plants (e.g., cotton and wheat) over several decades as farmland. SOC contents ranges from about 0.7% in the surface horizon to 0.2% 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, stable isotopes composition 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 plant provides a strong isotopic tracer for probing the translocation of the sorghum residues in the soil system.

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. (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, two from the wet side and two from the 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 and 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 6N HCl hydrolysis were deeply 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. 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 and rinsed with DI water, 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 deionized (DI) water free of salt, and dried on a hot plate at 60-70°C. When dry, the soil sample was ground with a mortar and pestle, and examined again under a 20x microscope to remove any remaining identifiable plant fragments.

Soil pool separation

Acid hydrolysis with 6N 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 g of soil were placed into a 500 ml round-bottom flask with 150 ml of 6N HCl. With a water-cooled condenser installed above the flask, the mixture was heated to boiling with an electric heating mantle for ca. 18 hours. After hydrolysis, the mixture was allowed to cool down and centrifuged at 2500 rpm for 15-20 minutes. Supernatant liquid was decanted to a pre-weighed 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 pre-weighed vials. Both the 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, and analyzed for carbon and δ$^{13}$C.
Carbon content and stable-isotope analysis

Whole soil and soil fractions (labile and recalcitrant pools) were analyzed for total C content (C%) 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 to remove carbonates. Whole soil was analyzed on a Finnigan-MAT Delta S dual inlet gas source isotope ratio mass spectrometer in 2000 by injecting pure CO$_2$ prepared off-line into the mass spectrometer. To convert soil organic carbon 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 two hours and at 650 °C for another two hours, according to the procedures described by Boutton (1991) and Leavitt et al. (in revision). 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” 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 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 which 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. ±0.006‰ for repeated analysis of the same organic standard samples.

*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 thousand (‰). The equation for calculation of \( \delta^{13}C \) is described as:

\[
\delta^{13}C(‰) = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 10^3
\]

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 (\( f_{\text{new}} \)) was calculated with the isotopic mixing model (Leavitt et al., 1994) as:

\[
f_{\text{new}} = \frac{\delta^{13}C_{\text{sample}} - \delta^{13}C_{\text{old}}}{\delta^{13}C_{\text{new}} - \delta^{13}C_{\text{old}}}
\]

where, \( \delta^{13}C_{\text{sample}} \) and \( \delta^{13}C_{\text{old}} \) are the isotopic signatures of SOC after and before the sorghum FACE experiment, respectively. \( \delta^{13}C_{\text{new}} \) 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.

The fraction of original (old) SOC was calculated as: \( f_{\text{old}} = 1 - f_{\text{new}} \). Here, we assumed all SOC after two-year FACE experiment had only two sources: remaining original SOC and inputs of new sorghum residues.

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

\[
C_{\text{new}} \% = f_{\text{new}} \times C_{\text{total}} \quad \text{and} \quad C_{\text{old}} \% = f_{\text{old}} \times C_{\text{total}}
\]

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

\[
X_t = X_0 \ e^{-kt}, \quad \text{and}
\]
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. 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. Evidence from other studies (Leavitt et al., 1996; Paul et al., 2001a) indicates the MRT of labile C is much younger than that of bulk SOC and recalcitrant C is much older than bulk SOC.

**Statistical Analysis**

The significance of CO$_2$ and water effects was tested by analyses of variance (ANOVA) and paired-t test. A one-way ANOVA was used to test CO$_2$ or water effects on total SOC, labile and recalcitrant C, and $C_r/C_{\text{total}}$ and $C_l/C_{\text{total}}$ ratios. A two-way ANOVA was used to test the main effects of CO$_2$ and water on SOC decomposition, and organic C content in labile and recalcitrant pools. A student t-test was applied to test the difference significance of separated CO$_2$ and water effects on all resulting parameters. To assess the relationships between MRT of bulk SOC and ratios of $C_r$ and $C_l$ to $C_{\text{total}}$, 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 were standard deviation. Statistically significant differences were considered at the $\alpha = 0.05$ probability level. All statistical procedures were conducted using Minitab statistical software (Version 13, Silicon Graphics).

Results and Discussion

**Labile and recalcitrant pools**

Not surprisingly, we did not find significant differences in averaged total SOC ($C_t$) between elevated and ambient CO$_2$ treatments after the two-year FACE experiment, but we did detect the significant shifts of SOC between labile and recalcitrant C pools (Table 1). Viewed across all treatments, elevated CO$_2$ significantly (one-way ANOVA: $p < 0.05$) increased averaged recalcitrant carbon ($C_r$) content in the soil profile from 1.35 g kg$^{-1}$ in CD to 1.56 in FD, and from 1.70 g kg$^{-1}$ in CW to 1.80 g kg$^{-1}$ in FW. In contrast, averaged labile carbon ($C_l$) content was significantly reduced (one-way ANOVA: $p < 0.05$) from 1.83 g kg$^{-1}$ and 1.71 g kg$^{-1}$ in CD and CW to 1.33 g kg$^{-1}$ and 1.34 g kg$^{-1}$ in the FD and FW plots, respectively. The increase in $C_r$ and decrease in $C_l$ under elevated CO$_2$ resulted in significantly higher (paired t-test: $p < 0.01$) $C_r/C_t$ ratios (0.54 and 0.58 in FD and FW, respectively) and lower $C_l/C_t$ ratios (0.46 and 0.43 in FD and FW, respectively) than their Control treatments (the $C_r/C_t$ ratios of 0.42 and 0.50, and the $C_l/C_t$ ratios of 0.58 and 0.50 in CD and CW, respectively). Higher $C_r/C_t$ ratios and lower $C_l/C_t$ ratios reflect the tendency of SOC to be more stable under elevated CO$_2$ than ambient CO$_2$ treatment.
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 two-year C4-sorghum Free-air CO\(_2\) enrichment (FACE) experiment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Depth (cm)</th>
<th>C(_t) (g kg(^{-1}))</th>
<th>C(_l) (g kg(^{-1}))</th>
<th>C(_r) (g kg(^{-1}))</th>
<th>C(_r)/C(_t) ratio</th>
<th>C(_l)/C(_t) ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD</td>
<td>0-15</td>
<td>5.06±0.34</td>
<td>2.15±0.93</td>
<td>2.91±0.16</td>
<td>0.41±0.13</td>
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<td>15-30</td>
<td>4.25±0.50</td>
<td>2.08±0.66</td>
<td>2.44±0.19</td>
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<td>45-60</td>
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<td>1.33±0.71</td>
<td>1.15±0.01</td>
<td>0.52±0.14</td>
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<tr>
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<td>80-100</td>
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<td>0.72±0.13</td>
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<td></td>
<td>Average†</td>
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<td>1.35</td>
<td>1.83</td>
<td>0.42</td>
<td>0.58</td>
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<tr>
<td>FD</td>
<td>0-15</td>
<td>4.17±0.83</td>
<td>2.22±0.06</td>
<td>1.94±0.97</td>
<td>0.55±0.14</td>
<td>0.45±0.14</td>
</tr>
<tr>
<td></td>
<td>15-30</td>
<td>4.11±0.01</td>
<td>2.17±0.07</td>
<td>1.94±0.15</td>
<td>0.51±0.09</td>
<td>0.49±0.09</td>
</tr>
<tr>
<td></td>
<td>45-60</td>
<td>3.15±0.02</td>
<td>2.00±0.12</td>
<td>1.15±0.14</td>
<td>0.64±0.04</td>
<td>0.36±0.04</td>
</tr>
<tr>
<td></td>
<td>80-100</td>
<td>1.30±0.01</td>
<td>0.54±0.17</td>
<td>0.75±0.16</td>
<td>0.42±0.13</td>
<td>0.58±0.13</td>
</tr>
<tr>
<td></td>
<td>Average†</td>
<td>2.89**</td>
<td>1.56*</td>
<td>1.33*</td>
<td>0.54**</td>
<td>0.46**</td>
</tr>
<tr>
<td>CW</td>
<td>0-15</td>
<td>5.49±0.42</td>
<td>2.62±0.39</td>
<td>2.88±0.13</td>
<td>0.48±0.04</td>
<td>0.53±0.04</td>
</tr>
<tr>
<td></td>
<td>15-30</td>
<td>4.33±0.24</td>
<td>1.95±0.21</td>
<td>2.38±0.18</td>
<td>0.45±0.04</td>
<td>0.55±0.04</td>
</tr>
<tr>
<td></td>
<td>45-60</td>
<td>3.04±0.04</td>
<td>1.71±0.09</td>
<td>1.33±0.05</td>
<td>0.56±0.02</td>
<td>0.44±0.02</td>
</tr>
<tr>
<td></td>
<td>80-100</td>
<td>1.81±0.76</td>
<td>0.98±0.33</td>
<td>0.83±0.03</td>
<td>0.50±0.19</td>
<td>0.50±0.19</td>
</tr>
<tr>
<td></td>
<td>Average†</td>
<td>3.40</td>
<td>1.70</td>
<td>1.71</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>FW</td>
<td>0-15</td>
<td>5.61±0.81</td>
<td>3.18±0.42</td>
<td>2.43±0.04</td>
<td>0.57±0.02</td>
<td>0.43±0.02</td>
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<tr>
<td></td>
<td>15-30</td>
<td>3.82±0.24</td>
<td>2.08±0.44</td>
<td>1.74±0.20</td>
<td>0.58±0.15</td>
<td>0.42±0.15</td>
</tr>
<tr>
<td></td>
<td>45-60</td>
<td>2.63±0.31</td>
<td>1.71±0.22</td>
<td>1.07±0.15</td>
<td>0.59±0.11</td>
<td>0.41±0.11</td>
</tr>
<tr>
<td></td>
<td>80-100</td>
<td>1.54±0.38</td>
<td>0.67±0.28</td>
<td>0.87±0.11</td>
<td>0.43±0.07</td>
<td>0.57±0.07</td>
</tr>
<tr>
<td></td>
<td>Average†</td>
<td>3.13**</td>
<td>1.80**</td>
<td>1.44*</td>
<td>0.58*</td>
<td>0.46*</td>
</tr>
</tbody>
</table>

† Average---averaged SOC content (g kg\(^{-1}\)) of 4 depths adjusted with the soil bulk density and thickness of each layer were calculated as:

\[
C(\text{g kg}^{-1}) = \frac{\sum_{i=1}^{n} (L_i \cdot \rho_{bi} \cdot C_i)}{\sum_{i=1}^{n} L_i \cdot \rho_{bi}}, \text{ where, } L_i \text{ is thickness (cm) of } i\text{th layer}; \rho_{bi} \text{ is soil bulk density (g cm}^{-3}\text{) in } i\text{th layer}; C_i \text{ is soil organic carbon content (C: g kg}^{-1}\text{) at } i\text{th layer}; n=4. \text{ Statistical significance of differences between FACE and corresponding Control were considered as } *-- p < 0.05, **-- p < 0.01, \text{ and ns-- p > 0.05.}
The elevated CO$_2$ plus deficient-water supply treatment 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 conditions, average C$_r$ in FD was 1.56 g kg$^{-1}$, significantly higher (paired t-test: p = 0.021) than the CD site (1.35 g kg$^{-1}$), an increase of 15.6%. C$_l$ was 1.33 g kg$^{-1}$ in FD, significantly lower (paired t-test: p = 0.012) than the CD treatment (1.83 g kg$^{-1}$), a decrease of 27.3%. Under ample water conditions, however, elevated CO$_2$ slightly increased C$_r$ by 5.9% and decreased C$_l$ by 21.6%. For total SOC, ample-water supply resulted in slightly higher SOC content than deficient-water supply in both elevated and ambient CO$_2$ treatments compared to their Controls. These trends were consistent with the previous conclusions from this study that found in absolute terms, the great benefit from long-term CO$_2$ enrichment occurs when water supply is nonlimiting, but the enhancement of relative change in biomass increases substantially when water is deficient (Ottman et al., 2001).

In the soil profile, from the surface to 60 cm, the proportion of non-hydrolysable C to total SOC (C$_r$/C$_l$) generally increased (from 0.41 to 0.64) with depth, whereas the proportion of hydrolysable C to total SOC (C$_l$/C$_r$) decreased from 0.59 to 0.36 with 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$_r$ ratio unexpectedly increased whereas C$_r$/C$_l$ ratio decreased, indicating a large proportion of hydrolysable C was present in the deeper soil. This phenomenon perplexes us and explanations may include the organic matter contributions from deeper roots or the leaching of soluble organic C down to depth. Considering the
long turnover time at the depth, the large portion of labile C is probably mainly parent material-derived SOC. Compared with ambient CO$_2$ for the whole soil profile, elevated CO$_2$ increased $C_r/C_t$ on average by 0.12 and 0.08 in dry and wet sites, respectively, and reduced the $C_r/C_t$ by 0.12 and 0.04 in dry and wet sites, respectively. The largest effects of CO$_2$ on these ratios were found in the surface horizon (0-30 cm). For example, FW was significantly different in $C_r/C_t$ from CW only at 0-30 cm, but not below the 30 cm, indicating elevated CO$_2$ had a stronger effect on the dynamics of SOC at surface layer than in subsurface layers.

\[\delta^{13}C\text{ values}\]

The $\delta^{13}C$ values of labile and recalcitrant C in soil profiles were greatly affected by the input of new C$_4$-sorghum residues (Fig. 1). Compared to the $\delta^{13}C$ values of 1998 pre-experiment bulk SOC, the $\delta^{13}C$ values of both labile and recalcitrant C because significantly less negative ($p < 0.01$), suggesting $^{13}C$-rich sorghum residues were incorporated into both labile and recalcitrant pools. Sorghum, a C$_4$-plant, was $^{13}C$-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 (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 $\delta^{13}C$ values 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 native C, indicating
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 collected 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 grew since 1998.
that new sorghum residues were the major contributor to the labile C pool, whereas original SOC was a major component of the recalcitrant C pool.

For both pools, elevated CO$_2$ resulted in less negative $\delta^{13}$C than that of ambient CO$_2$, but significant differences ($p < 0.05$) between FACE and Control recalcitrant C pools and labile C pools were only found in surface soil horizon (0-30 cm)(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 wet and dry plots, the differences between FACE and Control were about 0.58‰ and 0.65‰. This difference was smaller than the difference in $\delta^{13}$C values between FACE and Control sorghum residues, indicating that a portion of native old SOC transformed into the labile C pool and diluted the $\delta^{13}$C values. 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 residues and more original SOC was contributed to this pool. For the recalcitrant C pool, $\delta^{13}$C values were very close to those of original SOC, meaning a major component of recalcitrant C pool was original old SOC. The difference in $\delta^{13}$C between FACE and Control was significant ($p < 0.05$) throughout the entire soil profile, implying CO$_2$ strongly affected the recalcitrant C pool, probably through effects on sorghum root growth and the change in residue chemistry that might have a long-term effect on SOC pools.

*New and old carbon translocation*

The translocation of new and old carbon among labile and recalcitrant pools was significantly influenced by elevated CO$_2$ (Table 2 and Fig. 2 and 3). Viewed across all
Table 2. Means ± standard deviation of total SOC, new and old labile C, and new and old recalcitrant C in the CD (Control+dry), FD (FACE+dry), CW (Control+wet), and FW (FACE+wet) soils from the depth of 0 to 100 cm. The data were the average of 3 replicates in the unit of kg per square meter. For each sample site, the data were calculated as:

\[ C(\text{kg/m}^2) = \sum_{i=1}^{n} (L_i \times \rho_{bi} \times C_i) / 10, \]

where, \( L_i \) is thickness (cm) of \( i \)th layer; \( \rho_{bi} \) is soil bulk density (g/cm\(^3\)) at the \( i \)th layer; \( C_i \) is organic C content (C%) of the \( i \)th layer; \( n=6 \). Here, because we did not analyze the soils collected from 30-45cm and 60-80cm, the data used in above equation for these two layers were inserted, using the average of the values of adjacent two layers.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total SOC (kg/m(^2))</th>
<th>Labile carbon (C(_i)) (kg/m(^2))</th>
<th>Recalcitrant carbon (C(_r)) (kg/m(^2))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>New C(_i)</td>
<td>Old C(_i)</td>
</tr>
<tr>
<td>CD</td>
<td>3.83 (0.46)</td>
<td>0.34 (0.04)</td>
<td>1.71 (0.06)</td>
</tr>
<tr>
<td>FD</td>
<td>4.02 (0.41)</td>
<td>0.28 (0.03)</td>
<td>1.52 (0.20)</td>
</tr>
<tr>
<td>FD/CD</td>
<td>1.05(^{\text{ns}})</td>
<td>0.81*</td>
<td>0.89*</td>
</tr>
<tr>
<td>CW</td>
<td>4.46 (0.39)</td>
<td>0.41 (0.05)</td>
<td>1.79 (0.02)</td>
</tr>
<tr>
<td>FW</td>
<td>4.26 (0.44)</td>
<td>0.37 (0.08)</td>
<td>1.51 (0.22)</td>
</tr>
<tr>
<td>FW/CW</td>
<td>0.96(^{\text{ns}})</td>
<td>0.90*</td>
<td>0.84*</td>
</tr>
</tbody>
</table>

Significant levels of difference between FACE and Control were considered as *---p <0.05; **---p < 0.01; ns--- p > 0.05.
treatments, we found that elevated CO\textsubscript{2} resulted in 25.0% and 36.4% more new C in dry and wet recalcitrant pools, respectively, and 17.6% and 9.8% less in dry and wet labile pools, respectively, compared to ambient CO\textsubscript{2} treatments, suggesting the carbon derived from sorghum residues transfers into the recalcitrant pool from the labile C pool (Table 2, and Fig. 2). These new C\textsubscript{r} carbon could be dominated by sorghum-residue-derived resistant compounds such as fats, waxes, resins, suberins, lignin, and non-lignin phenolics (Rovira and Vallejo, 2002), which confirmed our findings from the examination of sorghum chemistry that the resistant compounds of sorghum tissues such as lignin, and non-lignin phenolics increased with elevated CO\textsubscript{2} (increasing 7.0-3.4%, Cheng et al. in preparation). However, we found that the increment of new C in the recalcitrant C pool was much higher than the incremental chemical change of those resistant compounds in sorghum tissues. This may be explained by the possibility that the dominant effects of initial lignin and non-lignin phenolic concentrations in sorghum tissues were due not only to directly added resistant material to new soil C\textsubscript{r} but also involved the formation of other recalcitrant compounds that were synthesized by these chemical compounds with other labile compounds, such as humic substances (Campbell et al., 1967), which are the most stable against chemical and biological degradation (Kögel-knaber, 1993). 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-degraded compounds originating from sorghum residues such as amino acids and soluble carbohydrates, which also did not decline in sorghum tissues as much as in the labile soil C pool under elevated CO\textsubscript{2}. This further showed that under
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 (0-100cm) 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 pre-experiment SOC (C: dry and D: wet) at the soil profile (0-100cm) in FACE (square) and Control (diamond) plots. Error bars represent one standard deviation, n = 3.
elevated CO$_2$ labile carbon was probably transferred to the recalcitrant pool in the soil or degraded into CO$_2$ released from the soil.

Similar to new C, old C in the recalcitrant pool was also more resistant to degradation 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% greater (one-way ANOVA: p < 0.05) under elevated CO$_2$ with deficient water treatment and slightly higher with ample water treatment than their comparative Controls, whereas in the labile pool the average old C was 11.1% and 15.6% significantly lower (one-way ANOVA: p < 0.05) in FD and FW treatments, respectively, than their comparative Controls. This indicates that mineralization of native soil organic carbon was retarded under CO$_2$ enrichment probably because of the depression of soil microbe activity with more resistant substrate input under elevated CO$_2$ or alternatively, easily decomposable substrates stimulating microorganism activity to accelerate the decomposition of old SOC under ambient CO$_2$. This is consistent with our results from laboratory incubation study (Cheng et al., preparation), which show sorghum residues collected from elevated CO$_2$ were degraded more slowly than those derived from ambient CO$_2$. Again, this confirms that elevated CO$_2$ effects on dynamics of SOC depend more on plant residue quality than on quantity. Because most of the residue was input in surface soil (0-30cm), elevated CO$_2$ effects on the shift of labile and recalcitrant C pool most significantly occurred in this layer. (Table 2 and Fig. 2 and 3).
Mean Residence Time

The rate constant (k) for loss of bulk SOC in the surface soil was much higher than in subsurface soil, and consequently, average mean residence time (MRT) or turnover time of the SOC ranged from 12 to 58 yr for the surface horizon, much shorter than subsurface horizons where average MRT ranged from 153-185 yr (Table 3). This increase in MRT is consistent with other studies (Paul et al., 2001a; Paul et al., 2001b) using $^{14}$C showing that the MRT of SOC in US Midwestern agricultural soils increases significantly with depth. Compared to the ambient CO$_2$ treatment, at 0-30 cm, elevated CO$_2$ significantly reduced ($p < 0.05$) the decay rate (k) of SOC from 0.046-0.071 in CD to 0.017-0.065 in FD, and from 0.046-0.085 in CW to 0.018-0.058 in FW. At 30-60 cm, the decay rate was very low ranging from about 0.0054 and 0.0065, with no significant difference between elevated and ambient CO$_2$ treatment. As a result, MRT for bulk SOC at 0-30 cm was higher under elevated CO$_2$ than ambient CO$_2$, with a range of 15-58 yr and 17-57 yr in FD and FW, respectively, compared to 14-22 yr and 12-22 yr in CD and CW, respectively. At 30-60 cm the MRT was much longer but with no difference between elevated and ambient CO$_2$ treatment (Table 3).

The longer MRTs of SOC for FACE plots were 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. From the soil respiration study of this FACE experiment (Cheng et al. in preparation), we found that elevated CO$_2$ increased the proportion of soil respiration derived from root respiration or decomposition of new root materials by an average of 15%. This mechanism is consistent
Table 3. Averaged SOC decay rate \((k: \text{ yr}^{-1})\) and mean residence time (MRT: yr) of bulk soil organic matter at soil profile (0-60cm), as determined by \(\delta^{13}\text{C}\) values and soil organic carbon (SOC) content (C: mean ±1 standard deviation, g kg\(^{-1}\)) in 1998 and 1999 FACE experiments, in which free-air CO\(_2\) enrichment (FACE: F, 560 µmol mol\(^{-1}\)) and ambient CO\(_2\) (Control: C, 360 µmol mol\(^{-1}\)) with wet (W) and dry (D) regimes were treatments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Depth</th>
<th>SOC (g kg(^{-1}))</th>
<th>Fraction of 1998 C in 1999 SOC</th>
<th>k (yr(^{-1}))</th>
<th>MRT (yr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(cm)</td>
<td>1998</td>
<td>1999</td>
<td>(f)</td>
<td></td>
</tr>
<tr>
<td>CD</td>
<td>0-15</td>
<td>7.06±1.59</td>
<td>6.54±0.22</td>
<td>0.937</td>
<td>0.0710</td>
</tr>
<tr>
<td></td>
<td>15-30</td>
<td>5.76±1.02</td>
<td>5.90±1.16</td>
<td>0.888</td>
<td>0.0460</td>
</tr>
<tr>
<td></td>
<td>30-60</td>
<td>4.01±0.73</td>
<td>4.10±0.48</td>
<td>0.967</td>
<td>0.0054</td>
</tr>
<tr>
<td>FD</td>
<td>0-15</td>
<td>7.03±0.71</td>
<td>6.93±0.99</td>
<td>0.890</td>
<td>0.0652</td>
</tr>
<tr>
<td></td>
<td>15-30</td>
<td>5.35±0.89</td>
<td>5.60±0.24</td>
<td>0.923</td>
<td>0.0174</td>
</tr>
<tr>
<td></td>
<td>30-60</td>
<td>4.12±0.89</td>
<td>4.21±0.84</td>
<td>0.966</td>
<td>0.0058</td>
</tr>
<tr>
<td>CW</td>
<td>0-15</td>
<td>7.28±0.80</td>
<td>6.71±0.55</td>
<td>0.916</td>
<td>0.0846</td>
</tr>
<tr>
<td></td>
<td>15-30</td>
<td>5.87±0.77</td>
<td>5.69±0.38</td>
<td>0.941</td>
<td>0.0458</td>
</tr>
<tr>
<td></td>
<td>30-60</td>
<td>4.28±0.26</td>
<td>4.28±0.54</td>
<td>0.987</td>
<td>0.0065</td>
</tr>
<tr>
<td>FW</td>
<td>0-15</td>
<td>6.91±0.62</td>
<td>7.18±0.35</td>
<td>0.856</td>
<td>0.0589</td>
</tr>
<tr>
<td></td>
<td>15-30</td>
<td>5.40±0.95</td>
<td>5.85±0.70</td>
<td>0.890</td>
<td>0.0176</td>
</tr>
<tr>
<td></td>
<td>30-60</td>
<td>4.20±0.49</td>
<td>4.30±0.34</td>
<td>0.965</td>
<td>0.0058</td>
</tr>
</tbody>
</table>
with the hypotheses of (Goudriaan and de Ruiter, 1983) that proposed that increased
inputs of soluble, easily decomposed C resulting from elevated atmospheric CO₂ 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 CO₂ and decrease in
turnover time of native SOC has been broadly reported by many other researchers
(Chaudhuri et al., 1986; Goudriaan and de Ruiter, 1983; Rogers and Prior, 1992; Van
Veen et al., 1991). Second, after the growing season, above-ground biomass of sorghum,
except for grain, were returned to the soil. Those new soil organic materials contained
more secondary and carbon-based structural compounds when sorghum was grown under
elevated CO₂. These lower quality residues may reduce energy supplied to
microorganisms and consequently reduced microbial activity in FACE soils (this
conflicts with the findings of Rilling 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 the MRT of FACE soil organic carbon increased. This
mechanism conforms to studies by other researchers who have demonstrated that
elevated CO₂ reduces substrate quality and increases MRT of SOC. (Berendse et al.,
1987; Lewis and Yamamoto, 1990; McLaugherty and Berg, 1987; Nicolai, 1988).

Correlation MRT with C pools

As mentioned, we could not calculate the MRT of each C pool because of the lack
of data from 1998 soil. We only calculated the MRT of bulk SOC for the soil collected at
the beginning of 1998 and at the end of 1999 growing season. As noted, the values of
bulk SOC (Table 3) determined by dry combustion methods (Leavitt et al., in revision) were slightly higher (about average 1.6%) than the total C from the sum of each C pool (Table 1). This difference may be attributed to the different procedures, one involving mass spectrometric methods and the other using manometric methods. Previous studies by Leavitt et al. (1996) and Paul et al. (2001a) have shown that carbon in labile pools is younger than bulk SOC, whereas carbon in recalcitrant pools is older than bulk SOC. To confirm these findings with our results, 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 MRT and $C_r/C_t$ ratio, the portion of recalcitrant carbons in total SOC, and a significant negative correlation ($r^2 = 0.36$, $P < 0.05$) between MRT and $C_l/C_t$ ratio, the portion of labile C in total SOC, 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) reported that total SOC has an MRT calculated from $^{14}C$ that is 176 times as large as that indicated by $\delta^{13}C$. 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.
Fig. 4 Regression of bulk SOC MRT with the ratios of $C_r/C_t$ (squares) and $C_i/C_t$ (triangles)

$R^2 = 0.3621 \quad p=0.039$
Conclusion

Stable-carbon isotopic tracing ($\delta^{13}$C) was used to probe 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 (Cotrufo et al., 1998; Heal et al., 1997), 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. Overall, in our study, quantification of labile and recalcitrant carbon pools clearly demonstrates 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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