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Soil organic matter dynamics in grassland soils under elevated CO₂: Insights from long-term incubations and stable isotopes

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Abstract

Elevated atmospheric carbon dioxide (CO₂) levels generally stimulate carbon (C) uptake by plants, but the fate of this additional C largely remains unknown. This uncertainty is due in part to the difficulty in detecting small changes in soil carbon pools. We conducted a series of long-term (170–330 days) laboratory incubation experiments to examine changes in soil organic matter pool sizes and turnover rates in soil collected from an open-top chamber (OTC) elevated CO₂ study in Colorado shortgrass steppe. We measured concentration and isotopic composition of respired CO₂ and applied a two-pool exponential decay model to estimate pool sizes and turnover rates of active and slow C pools. The active and slow C pools of surface soils (5–10 cm depth) were increased by elevated CO₂, but turnover rates of these pools were not consistently altered. These findings indicate a potential for C accumulation in near-surface soil C pools under elevated CO₂. Stable isotopes provided evidence that elevated CO₂ did not alter the decomposition rate of new C inputs. Temporal variations in measured δ¹³C of respired CO₂ during incubation probably resulted mainly from the decomposition of changing mixtures of fresh residue and older organic matter. Lignin decomposition may have contributed to declining δ¹³C values late in the experiments. Isotopic dynamics during decomposition should be taken into account when interpreting δ¹³C measurements of soil respiration. Our study provides new understanding of soil C dynamics under elevated CO₂ through the use of stable C isotope measurements during microbial organic matter mineralization.

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Keywords: Soil carbon pools; Soil organic matter; Mean residence time; Turnover rate; ¹³C/¹²C; CO₂ enrichment; Carbon cycle; Global change; Decomposition; Isotopic fractionation; Incubation; Respiration; Microbial biomass

1. Introduction

Atmospheric carbon dioxide (CO₂) concentrations are rising by about 1% per year, owing to anthropogenic emissions, and are expected to double in the coming century (IPCC, 2001). Increasing atmospheric CO₂ affects terrestrial ecosystems because it has the potential to stimulate plant growth, particularly in C3 plants. In the shortgrass steppe of northeastern Colorado, above- and belowground growth were both enhanced by 15–35% (depending on precipitation) during a 5-year open-top chamber (OTC) experiment (Morgan et al., 2004; Milchunas

et al., 2005). Grasslands exposed to elevated CO₂ have shown increased belowground C inputs and may have increased soil organic matter (SOM) storage (Jastrow et al., 2000; Pendall et al., 2004b). However, the dynamics and fate of SOM pools remain uncertain.

Carbon cycling rates are frequently stimulated in ecosystems that have been experimentally exposed to elevated concentrations of atmospheric CO₂ because plant growth and soil respiration rates are generally both stimulated (Hungate et al., 1997; Pendall et al., 2003). If the residence or turnover times of SOM pools change, the rate of nitrogen (N) cycling and N availability may also be affected, providing a possible feedback to plant growth which could be positive or negative (Diaz et al., 1993; Hu et al., 1999). For example, increased C:N ratio in plant

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residues grown under elevated CO₂ may contribute to progressive N limitation of future plant growth (Luo et al., 2004). On the other hand, increased labile substrates belowground may stimulate decomposition of older SOM and thereby enhance N mineralization (Zak et al., 1993). Furthermore, residence times of various SOM pools are key parameters in ecosystem models, so understanding the sensitivity of residence times to global changes such as increased atmospheric CO₂ is critical to building realistic models.

Although SOM is comprised of an array of compounds with widely varying decomposition dynamics, it may be conceptually divided into two or three compartments which decompose rapidly, slowly, or on timescales of centuries. These compartments have been called the active, slow, and passive (or resistant) pools, respectively (e.g., Schimel et al., 1985; Parton et al., 1987; Christensen, 1996). Long-term net changes in C sequestration resulting from elevated CO₂ must be mediated by slow-turnover pools, those with mean residence times (MRTs) of decades or longer. Predictive understanding of C cycle responses to global change therefore requires knowledge of the sizes and turnover rates of specific identifiable SOM pools. Long-term soil incubations provide a functional way to quantify pool sizes and turnover rates (Paul et al., 2001, 2006), allowing insight into effects of manipulative experiments on soil properties not apparent from field measurements.

In systems where the $\delta^{13}\text{C}$ value of plant-derived C contrasts with that of the pre-existing SOM pools, the isotopic offset, or disequilibrium, provides a way to trace inputs and efflux of newly fixed C to and from the soil. For example, fossil fuel-derived CO₂ with a $\delta^{13}\text{C}$ value of -40‰ was mixed with background air of -8‰ and imparted a tracer to a free-air CO₂ enrichment (FACE) wheat experiment that allowed detection of about 5% greater inputs of new C into belowground pools than under ambient conditions (Leavitt et al., 2001). These new C inputs to soils have been termed “rhizodeposition,” implying that turnover of roots and root exudates is the main contributor (Cardon et al., 2001; Pendall et al., 2004a, b). In our Colorado grassland OTC experiment, changes in $\delta^{13}\text{C}$ values allowed detection of increased decomposition rates (by 25–85% depending on soil moisture; Pendall et al., 2003), and doubling of rhizodeposition rates under elevated CO₂ (Pendall et al., 2004b). These results suggest that turnover rates and sizes of soil C pools increased as a result of exposure to elevated CO₂. If the additional C allocated belowground under elevated CO₂ experiences higher turnover rates compared to ambient CO₂ conditions, the potential for long-term C sequestration in soil may be limited.

The isotopic composition of respired CO₂ also can provide insights into decomposition dynamics (Ehleringer et al., 2000; Crow et al., 2006). The relative contributions of microbial and root respiration to total soil respiration have been estimated in systems where a shift in plant functional group from C3 to C4 photosynthetic pathway

has occurred (Rochette and Flanagan, 1997) or where ¹³C-depleted CO₂ has been used in elevated CO₂ experiments (Lin et al., 1999; Pendall et al., 2001, 2003). In many natural (non-labeled) systems, the $\delta^{13}\text{C}$ of SOM in soil profiles is higher than that of fresh litter, an effect that is stronger at higher temperatures (Garten et al., 2000) or with faster decomposition rates (Feng, 2002). Some studies have suggested that ¹³C enrichment occurs during organic matter decomposition (Mary et al., 1992; Schweitzer et al., 1999; Šantrůčková et al., 2000), whereas other studies have not supported the findings of fractionation (Wedin et al., 1995; Connin et al., 2001). If isotopic fractionation occurs during decomposition, the isotopic composition of respired CO₂ would differ from that of the remaining SOM, leading to isotopic disequilibrium even in systems that have not received a label or experienced a change in vegetation. If unaccounted for, disequilibrium of $\delta^{13}\text{C}$ between respired CO₂ and SOM due to either fractionation or vegetation change could challenge assumptions made to partition soil respiration fluxes (e.g., Pendall et al., 2003) and determine C source/sink relationships at global scales (e.g., Ciais et al., 1995).

We conducted a series of laboratory incubation experiments to quantify changes in pool sizes and turnover rates of active and slow organic matter pools in shortgrass steppe soils exposed to elevated CO₂ for 1–3 years. Furthermore, we measured $\delta^{13}\text{C}$ of respired CO₂ to evaluate changes in pool sizes and turnover rates of organic matter added since the beginning of the experiment and to examine the possibility of isotopic fractionation during decomposition. We hypothesized that active pool C and its turnover rate would increase but that slow pool C would be less affected, and that these changes would be soil depth-dependent. We also expected that the $\delta^{13}\text{C}$ of respired CO₂ would be different from that of total SOM, and that changes in $\delta^{13}\text{C}$ of CO₂ respired during decomposition would allow us to track the fate of the new belowground C inputs.

2. Methods

2.1. Site description and field methods

An elevated CO₂ experiment was conducted in the shortgrass steppe region of northeastern Colorado, at the USDA-ARS Central Plains Experimental Range (CPER; 40°40'N, 104°45'W), about 55 km northeast of Fort Collins, CO. The most abundant species at the study site were a C4 grass, *Bouteloua gracilis* (H.B.K.) Lag. (blue grama), and two C3 grasses, *Stipa comata* Trin and Rupr. (needle-and-thread grass) and *Pascopyrum smithii* (Rydb.) A. Love (western wheatgrass). Root biomass (including crowns) is responsible for ~70% of net primary production (NPP) in this ecosystem (Milchunas and Lauenroth, 2001). The soil at the experimental site is a Remmit fine sandy loam (Ustollic camborthid). Gravimetric water content of

this sandy soil is 18% at field capacity and 4% at the permanent wilting point.

Beginning in 1997 and ending in 2001, OTCs (4.5 m diameter) were used to evaluate the effects of elevated CO₂ on the shortgrass steppe ecosystem, with three replicate chambers at ambient (360 ± 20 ppmv) and at elevated (720 ± 20 ppmv) CO₂. Three non-chambered plots of the same area allowed evaluation of chamber effects. The three treatments are referred to as AC (ambient CO₂ chambers), EC (elevated CO₂ chambers), and NC (non-chambered plots). Chambers were placed on the plots before growth started in late March or early April and removed at the end of the growing season in late October. Blowers with ambient or elevated CO₂ ran continuously and resulted in average wind speeds similar to outside the chambers. The tank gas used to double the atmospheric CO₂ concentration produced air in the elevated chambers with a δ¹³C value of -24.7 ± 1.4‰, which differed from background air δ¹³C values of -8.1 ± 0.2‰ (Pendall et al., 2003). The experimental and chamber design were described in detail by Morgan et al. (2001).

Two soil cores (40-cm deep; 15-cm diameter) were collected from each plot at the end of the 1997, 1998, and 1999 growing seasons, after 1, 2, and 3 years of experimental treatments. By the time of sampling (October) in the shortgrass steppe, grasses had senesced and soil moisture was near the wilting point (5–9% volumetric soil moisture). Cores were collected volumetrically and divided into five depth increments (0–5, 5–10, 10–20, 20–30, and 30–40 cm). Gravel >2 mm and most roots were removed immediately after sampling, with additional fine-root picking done by hand on air-dried samples.

2.2. Laboratory methods

We consider each year's samples (1997–1999) as a separate laboratory experiment, referred to as the 1997, 1998 and 1999 experiments, respectively. Soil field replicates collected in 1997 were kept as separate laboratory replicates, whereas soil samples collected in 1998 and 1999 were inadvertently composited and therefore had to be sub-sampled for the incubation experiments. The statistical approach to account for the lack of true field replicates in the 1998 and 1999 experiments is described below. In the laboratory, 20 g of air-dried soil from the 5–10 cm ("topsoil") and 30–40 cm ("subsoil") depths were placed in disposable, polystyrene beakers which had small holes punched into the bases and pre-combusted glass fiber filters lining the bottoms for drainage (Townsend et al., 1997). The soils were wetted with deionized water to bring them to 80% of field capacity (~16% by weight) and gently compressed to simulate field bulk density. Each beaker was placed into a 500-ml canning jar with a lid modified to hold a 1.5-cm long, blue butyl rubber stopper. The 1997 soils were not placed into the plastic beakers or packed to field density. The jars were evacuated and flushed twice with dry air from a tank (370 ppm CO₂). The jars were

stored in a dark cabinet. The 1997 and 1998 soils were incubated at 20.5 ± 1 °C and the 1999 soils at 22 ± 1 °C. Headspace samples (8 ml) were collected in glass syringes fitted with gas-tight valves after mixing about 20% of the total volume by plunging the syringe up and down. Samples were collected at intervals ranging from 1 to 20 days (see Fig. 1 for sampling intervals). All incubation jars were flushed and refilled with dry tank air (370 ppm CO₂) following each sampling. Four blank jars (no soil) were included in each experiment to control for background CO₂ concentration. Each experiment was conducted until the rate of microbial respiration declined to near-steady-state

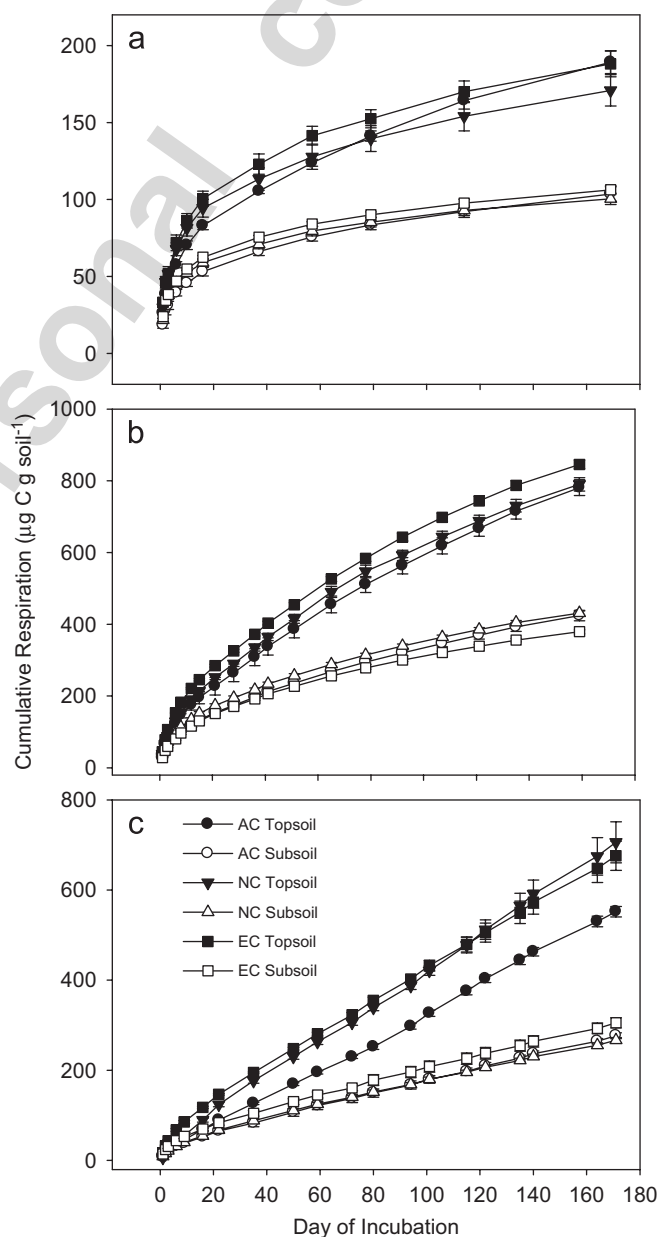


Fig. 1. Cumulative C mineralization in the three incubation experiments. Treatments are abbreviated as ambient CO₂ chamber (AC), elevated CO₂ chamber (EC), and non-chambered (NC) plots. (a) 1997 experiment, (b) 1998 experiment, and (c) 1999 experiment. Error bars are standard error.

values. The 1997 soils were incubated for 170 days, the 1998 soils for 330 days, and the 1999 soils for 295 days.

Headspace samples were analyzed for CO₂ concentration using an infrared gas analyzer, calibrated with four standard gases, with a precision of $\pm 3 \mu\text{mol mol}^{-1}$ over the concentration range 360–8000 $\mu\text{mol mol}^{-1}$ (Model LI-6251, LICOR Inc., Lincoln, NE). Most samples ranged between 500 and 2000 $\mu\text{mol mol}^{-1}$. Headspace samples were analyzed for stable isotopes of CO₂ using gas chromatography–isotope ratio mass spectrometry (GC–IRMS; Isoprime model, Micromass, UK; Miller et al., 1999). To ensure a linear response of the mass spectrometer, sample sizes varying from ~ 7 to $\sim 250 \mu\text{l}$ of soil gas were injected into a carrier gas stream, which was further split before being introduced into the mass spectrometer. This control of the sample size produced sample peak heights that were within $\sim 10\%$ of the peak height of the standard, and precision for $\delta^{13}\text{C}$ was better than $\pm 0.1\%$.

Total organic C content was determined on a Europa Scientific ANCA-NT System (PDZ Europa, Crew, England) as described in Pendall et al. (2004b). The resistant C pool (C_r) was estimated as non-hydrolyzable C (NHC) on a subset of samples at the end of the field experiment in 2001 by 6-N HCl hydrolysis (Paul et al., 2001). We assumed that NHC reflected C_r and that its pool size did not change over the duration of the experiment.

2.3. Calculations and statistics

We determined the turnover rates and pool sizes for active and slow C pools using PROC NLIN (SAS v. 9.1, SAS Institute Inc., 2003) with the following model (Paul et al., 2001):

$$\frac{dC}{dt} = C_a e^{-(k_a t)} + C_s e^{-(k_s t)}, \quad (1)$$

where dC/dt was the daily C mineralization rate, C_a and C_s were the active and slow pool sizes, and k_a and k_s were the

active and slow pool turnover rates, respectively. MRTs were calculated as the inverse of turnover rates. We constrained this two-pool model by assuming that C_s was the total soil C minus the resistant C (NHC) and active C pools and that the resistant pool had a turnover rate of > 1000 years. The turnover rate of the resistant pool was not found to influence results of faster pool sizes or kinetics (Paul et al., 2001).

The $\delta^{13}\text{C}$ values of respired CO₂ were used to estimate the proportion of “new” C (incorporated to the soil since the beginning of the experiment) utilized by microbes in topsoil (not subsoil). We applied a simple mass-balance approach:

$$\text{Proportion new C} = \frac{(\delta^{13}\text{C}_{\text{CO}_2} - \delta^{13}\text{C}_{\text{SOM}})}{(\delta^{13}\text{C}_{\text{roots}} - \delta^{13}\text{C}_{\text{SOM}})}, \quad (2)$$

where “new” $\delta^{13}\text{C}$ inputs from plants were determined from biomass-weighted $\delta^{13}\text{C}$ measurements of C3 and C4 roots. Pre-existing SOM ($\delta^{13}\text{C}_{\text{SOM}}$) values were measured on three replicate soil samples collected at the end of each growing season (Pendall et al., 2004b). The proportion of new C was plotted over time for all experiments, but large variability in the 1997 and 1998 results indicated insufficient isotopic labeling, so only proportions from 1999 are presented. The proportion of new C respired was also multiplied by the cumulative C respired during the 1999 experiment to demonstrate the amount of new C respired (Table 1). This approach assumed that fractionation during decomposition is minor compared to the larger differences between new inputs and older SOM. Microbial respiration of labile substrates thus would be isotopically similar to the new end member ($\delta^{13}\text{C}_{\text{roots}}$).

We evaluated possible shifts in $\delta^{13}\text{C}$ of SOM pools (active, slow, and total) for topsoils. We considered the active pool C to be characterized by CO₂ evolved over the first 6 days of the incubation period, after correction for the contribution of slow pool CO₂. The slow pool isotopic composition ($\delta^{13}\text{C}_s$) was defined by averaging $\delta^{13}\text{C}$ of CO₂

Table 1
Cumulative amount of C respired from soils after 170 days of incubation

	Topsoil				Subsoil			
	AC	EC	NC	EC/AC	AC	EC	NC	EC/AC
$\mu\text{g C g soil}^{-1}$								
1997	201 (7)a	202 (8)a	183 (11)a	1.00	109 (5)a	111 (3)a	104 (5)a	1.02
1998	829 (24)b	900 (13)a	838 (20)b	1.09	450 (15)a	407 (6)b	466 (3)a	0.90
1999	552 (11)b	676 (32)a	706 (45)a	1.22	277 (6)a	305 (11)a	255 (6)a	1.10
1999 New*	164 (8.8)a	207 (20)b	356 (24)a	1.26				
% of total C								
1997	2.8	2.7	2.5	0.96	2.1	2.3	2.3	1.09
1998	10.7	11.6	10.3	1.08	8.0	7.2	8.2	0.80
1999	7.5	8.6	7.9	1.15	4.3	4.9	4.5	1.14
1999 New (% of mineralized C)	29 (2)b	30 (2)b	50 (1)a					

Units are $\mu\text{g C g soil}^{-1}$ (standard error), or percent of total soil C. For each year, treatments within a depth category followed by different letters were significantly different (ANOVA, $P \leq 0.05$).

evolved during the steady-state phase (from day 70 to 120 in the 1997 experiment and from day 100 to 200 in the 1998 and 1999 experiments). The slow pool turnover rate and its isotopic signature were used to correct the active pool C signature ($\delta^{13}C_a$)

$$\delta^{13}C_{a+s6} \times CO_{2a+s6} = \delta^{13}C_a \times C_{a6} + \delta^{13}C_s \times C_{s6}. \quad (3)$$

For our incubation experiments, $\delta^{13}C_{a+s6}$ was the average $\delta^{13}C$ value in the first 6 days of the incubation; CO_{2a+s6} was the sum of CO_2 -C evolved in the first 6 days; C_{s6} was the amount of slow pool C evolved in the first 6 days, estimated as $MRT/6 \text{ days} \times C_s$; and C_{a6} was the amount of active pool C evolved in the first 6 days, estimated as $CO_{2a+s6} - C_{s6}$. Eq. (3) was used to solve for $\delta^{13}C_a$.

Pseudoreplication can invalidate ANOVA comparisons if it leads to a reduction in the variance relative to true replication (Hurlbert, 1984). In consultation with a statistician (S. Wolfe), we evaluated whether we could test for treatment effects within the 1998 and 1999 experiments, despite having composited the field replicates, by comparing the variance of each response variable (cumulative respiration, C_a , C_s , k_a , k_s) to that from the 1997 experiment (Sokal and Rohlf, 1995). Levene's test demonstrated homogeneity of variance within each experiment when samples were grouped by depth (the factor associated with most variability) (SAS Institute Inc., 2003). *F*-tests for each parameter comparing the 1997 with the 1998 and 1999 experiments revealed that variance was not significantly reduced by pseudoreplication except in the 1998 experiment for the turnover parameters (k_a and k_s), and therefore these parameters were not analyzed statistically for the 1998 experiment. For all other parameters and for all years, we evaluated differences in C pool sizes and turnover rates using two-way ANOVA with CO_2 treatment and depth as the groups within each experiment (1997–1999). We performed no statistical comparisons among the three experiments because the field and laboratory conditions differed by year. Each incubation jar was used as a replicate ($n = 6$ for 1997 experiment, $n = 4$ for 1998 and 1999 experiments), and results were considered significant if α was $\geq 95\%$. One-way ANOVA was used to determine treatment effects on $\delta^{13}C$ of different pools.

3. Results

3.1. Carbon mineralization

Cumulative C mineralization observed for all three experiments is shown in Fig. 1. We calculated total mineralizable C in the first 170 days of each incubation experiment for comparison among treatments, however, we make no direct comparisons among the different experiments (Table 1). For all three experiments, mineralizable C was higher in the topsoil (5–10 cm depth) than in the subsoil (30–40 cm depth; $P < 0.05$). In the 1998 and 1999 experiments, 9% and 22% more C was mineralized from EC topsoil than from AC topsoil, respectively ($P < 0.05$).

A chamber effect was noted in 1999, when C mineralization in the NC treatment was higher than in AC in the topsoil (Fig. 1; Table 1; $P < 0.05$). Mineralizable C made up 2–12% of total soil organic C (SOC) regardless of the treatment (Table 1). Mineralization rates always started very high and then decreased exponentially (Fig. 2). The initial, “active pool phase” took place for about the first 6–10 days. The longer “slow pool phase,” when mineralization rates dropped to $< 2\%$ of initial rates and leveled off to an approximate steady state, began at approximately day 16. Daily mineralization rates were used to calculate pool sizes and turnover rates from the two-pool model. The slopes of the curves during the active and slow pool phases

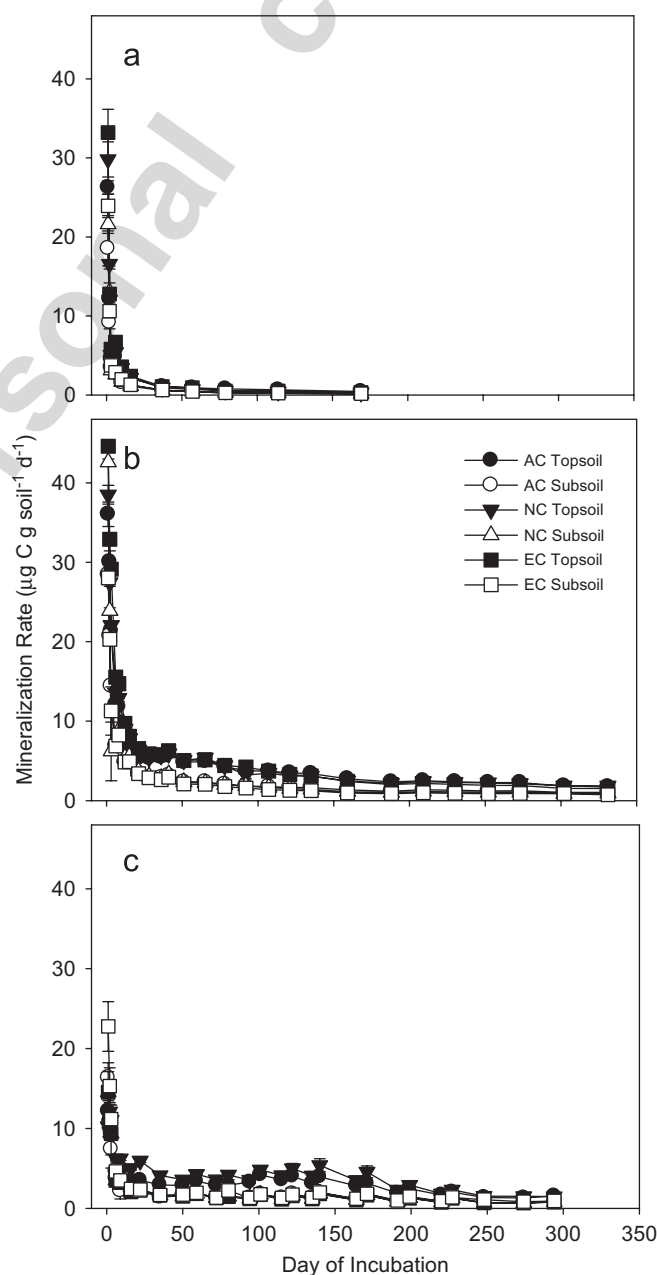


Fig. 2. Rate of CO_2 respiration from soil incubation experiments for: (a) 1997, (b) 1998, and (c) 1999 experiments. Abbreviations as in Fig. 1. Error bars are standard error.

approximate their respective turnover rates, and the areas under those portions of the curve approximate the pool sizes (Eq. (1); Fig. 2).

3.2. Pool sizes and turnover rates

Active pool C was consistently higher in EC than AC topsoils for all three experiments ($P < 0.05$; Table 2). The enhancement attributed to elevated CO_2 was evaluated by taking the ratio of the mass of active pool C in EC compared to AC (EC/AC), and this ratio increased over the 3 years, from 1.26 to 1.36 to 2.60. The active pool was always a greater proportion of total C in EC than the other treatments (proportions not shown); for example, in the 1999 experiment active C represented 0.5% of total C in AC and NC topsoils but 1.5% of the total in EC topsoils. Active pool C was higher in EC than AC subsoil for the 1997 and 1999 experiments (Table 2). Slow pool C was higher in EC than AC topsoils for all three experiments, but it was lower in EC than AC subsoils for all three experiments (Table 2). The ratio of EC/AC in slow pool C

ranged from 1.02 to 1.16 in topsoils and from 0.85 to 0.99 in subsoils, but these values did not change consistently over time.

There were no consistent effects of elevated CO_2 on turnover rates of active or slow pool C. Turnover rates of topsoil active C (k_a) and slow C (k_s) were highest in EC and lowest in NC treatments in the 1997 experiment ($P < 0.05$; Table 3). There was a trend of higher k_s in EC compared to NC in topsoil in the 1998 and 1999 experiments as well. Turnover rates of active and slow pool C in subsoil were not affected by elevated CO_2 in any experiment (Table 3).

3.3. Isotopic composition of mineralized C

In the 1997 experiment, CO_2 respired from EC topsoil was strongly ^{13}C -depleted compared to that from AC and NC soils during the first 80 days (Fig. 3a). This change in $\delta^{13}\text{C}$ value was observed after just one season of exposure to elevated CO_2 during which new biomass production had a $\delta^{13}\text{C}$ value of -35‰ (Table 4). The topsoil $\delta^{13}\text{C}$ - CO_2 in the NC treatment remained near a steady-state value, while

Table 2
Pool sizes determined by incubation of soils from the shortgrass steppe OTC experiment

	Exp.	Topsoil C pools				Subsoil C pools			
		AC	EC	NC	EC/AC	AC	EC	NC	EC/AC
Total C (mg kg^{-1})	1997	7140 (65)a	7440 (176)a	7250 (93)a	1.04	5090 (40)a	4860 (72)a	4600 (97)a	0.95
	1998	7770 (113)a	7770 (50)a	8100 (126)a	1.00	5600 (110)a	5650 (88)a	5700 (82)a	1.01
	1999	7370 (67)a	7870 (114)a	8910 (173)a	1.07	6460 (107)a	6280 (88)a	5630 (100)a	0.97
Active C (mg kg^{-1})	1997	67.5 (1.1)b	85.2 (8.2)a	80.6 (4.4)a	1.26	50.7 (5.0)b	64.0 (3.1)a	62.3 (2.5)ab	1.26
	1998	160.5 (13.3)b	217.6 (2.9)a	169.9 (2.2)b	1.36	110.2 (2.9)b	115.9 (2.1)b	128.7 (1.1)a	1.05
	1999	37.7 (3.2)b	98.1 (13.8)a	49.9 (12.4)b	2.60	55.5 (2.8)b	81.0 (5.1)a	50.5 (6.5)b	1.46
Slow C (mg kg^{-1})	1997	3255 (1)c	3670 (8)a	3373 (4)b	1.13	2257 (5)a	1927 (3)b	1723 (2)c	0.85
	1998	3792 (13.3)c	3862 (2.9)b	4137 (2.2)a	1.02	2704 (2.9)b	2668 (2.1)c	2753 (1.1)a	0.99
	1999	3516 (3.2)c	4078 (13.8)b	5078 (3.0)a	1.16	3613 (2.8)a	3338 (2.9)b	2757 (6.5)c	0.92
Resistant C ^a (mg kg^{-1})	2001	3817	3690	3793	0.97	2786	2866	2818	1.03

Reading across rows within an experiment (Exp.), differences among treatments are shown by different lower case letters ($P \leq 0.05$) statistical tests were performed on the curve-fitting results from each incubation sample). Standard errors shown.

^aResistant C (NHC) was analyzed only at the end of the experiment (2001) and considered constant.

Table 3
Soil carbon pool turnover rates determined from incubation experiments

	Exp.	Topsoil			Subsoil		
		AC	EC	NC	AC	EC	NC
Active turnover rate (k_a ; d^{-1})	1997	0.90 (0.04)ab	1.10 (0.06)a	0.80 (0.05)b	0.80 (0.09)a	0.90 (0.07)a	0.8 (0.1)a
	1998	0.25 (0.02) ^a	0.21 (0.002) ^a	0.23 (0.004) ^a	0.31 (0.01) ^a	0.28 (0.01) ^a	0.48 (0.01) ^a
	1999	0.33 (0.06)a	0.37 (0.07)a	0.26 (0.02)a	0.44 (0.11)a	0.45 (0.07)a	0.39 (0.07)a
Slow turnover rate (k_s ; y^{-1})	1997	0.21 (0.002)ab	0.33 (0.130)a	0.20 (0.033)b	0.16 (0.038)a	0.22 (0.047)a	0.19 (0.033)a
	1998	0.44 (0.018) ^a	0.45 (0.009) ^a	0.42 (0.016) ^a	0.34 (0.018) ^a	0.30 (0.009) ^a	0.39 (0.003) ^a
	1999	0.36 (0.002)a	0.39 (0.033)a	0.32 (0.02)b	0.18 (0.003)a	0.21 (0.010)a	0.20 (0.009)a

Active and slow turnover rates were determined as exponents in the two-pool model (Eq. (1)). Note that active turnover rate was measured in day^{-1} while slow turnover rate was measured in year^{-1} .

^aVariance of k_a and k_s from 1998 experiment was reduced by pseudoreplication, and therefore treatment effects were not determined.

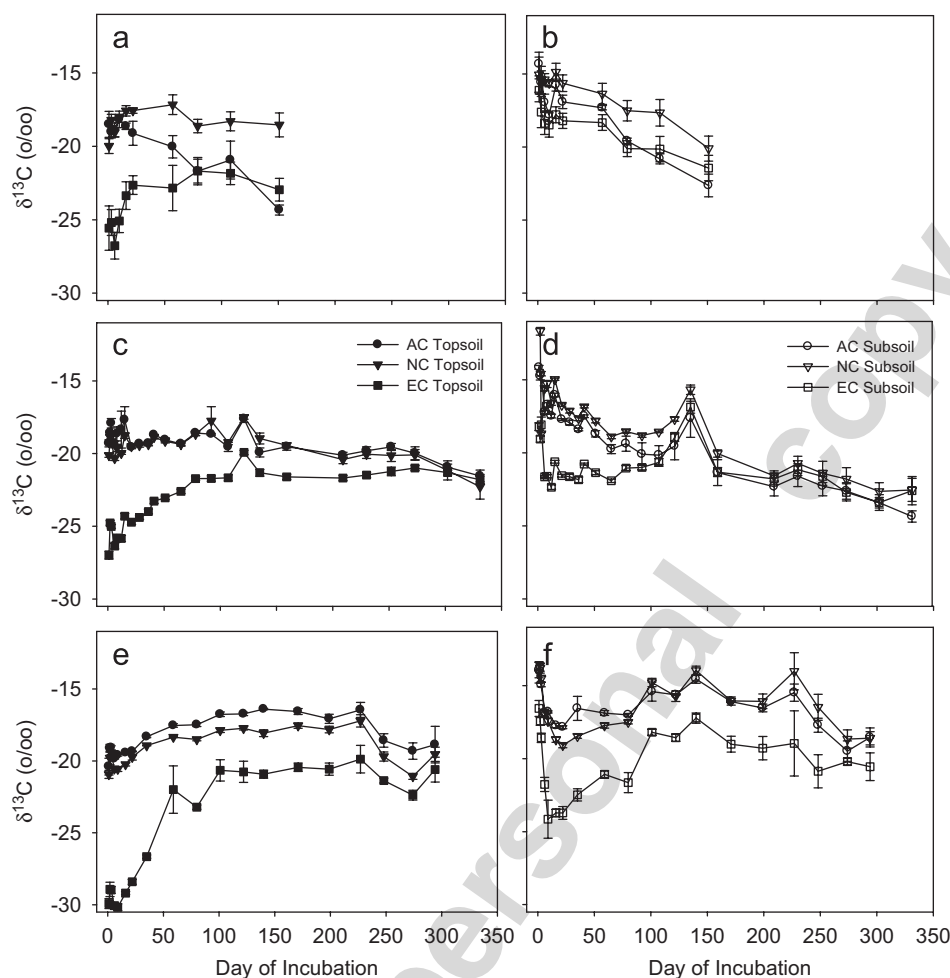


Fig. 3. Isotopic composition of respired CO_2 measured during incubation of: (a) 1997 topsoil, (b) 1997 subsoil, (c) 1998 topsoil, (d) 1998 subsoil, (e) 1999 topsoil, and (f) 1999 subsoil. Abbreviations as in Fig. 1. Error bars are standard error.

Table 4

$\delta^{13}\text{C}$ values of new C inputs from recent plant residue ($\delta^{13}\text{C}_{\text{roots}}$; also see Pendall et al., 2004b), active and slow pools in topsoils and total soil organic carbon (SOC) measured by EA-MS

Pool	1997			1998			1999		
	AC	EC	NC	AC	EC	NC	AC	EC	NC
Residue	-19.8 (1.3)a	-34.6 (1.2)b	-19.8 (1.3)a	-21.8 (2.0)a	-37.5 (3.0)b	-21.8 (2.0)a	-21.1 (0.60)a	-39.3 (2.1)b	-21.1 (0.60)a
Active	-18.5 (0.37)a	-25.7 (0.58)b	-19.2 (0.45)a	-18.8 (0.19)a	-25.8 (0.25)b	-19.6 (0.17)a	-19.6 (0.20)a	-29.5 (0.21)b	-20.2 (0.20)a
Slow	-20.4 (0.57)b	-22.0 (0.37)c	-17.5 (0.43)a	-19.1 (0.28)a	-21.1 (0.20)b	-18.8 (0.22)a	-16.6 (0.07)a	-20.7 (0.25)c	-17.8 (0.38)b
SOC	-16.5 (0.80)ab	-17.8 (0.10)b	-16.6 (0.70)a	-16.5 (0.50)a	-18.4 (0.30)b	-16.9 (0.30)a	-17.0 (0.30)a	-18.8 (0.30)b	-17.2 (0.50)a

Letters indicate differences among treatments (AC, EC, and NC) for each year for each pool. Standard error shown.

AC $\delta^{13}\text{C}$ - CO_2 declined over the 1997 incubation, and EC $\delta^{13}\text{C}$ - CO_2 increased to a plateau (Fig. 3a). The subsoil $\delta^{13}\text{C}$ - CO_2 declined steadily in all three treatments of the 1997 experiment (Fig. 3b). In the 1998 experiment, CO_2 from EC soils was depleted relative to that from AC and NC soils early in the incubation, and values converged after about 150 days for both topsoil and subsoil. $\delta^{13}\text{C}$ values of AC and NC CO_2 from the topsoil were similar and remained relatively constant during the incubation

(Fig. 3c). The $\delta^{13}\text{C}$ values of AC and NC CO_2 were also similar in the subsoil and declined over the incubation (Fig. 3d). In the 1999 experiment, $\delta^{13}\text{C}$ of EC CO_2 remained significantly lower than the other two treatments over the entire incubation for both topsoil and subsoil (Fig. 3e and f). In the 1998 and 1999 experiments, $\delta^{13}\text{C}$ values of CO_2 from topsoils remained fairly constant for all treatments between days 100 and 250, and declined thereafter (Fig. 3c and e). $\delta^{13}\text{C}$ of CO_2 produced from

subsoil of all treatments declined after about day 150 in the 1998 and 1999 experiments (Fig. 3d and f). Initially high $\delta^{13}\text{C}$ values in subsoil CO_2 (evolved during the first 3–4 days) were attributed to the presence of carbonates ($<0.1\%$).

The proportion of new (^{13}C -labeled) C respired from topsoil during the 1999 experiment (estimated from the 2-part mixing model, Eq. (2)), declined from 60–90% during the active pool phase to 10–30% during the slow pool phase (Fig. 4a). This proportion was used to calculate the cumulative new C respired over the course of the experiment (Fig. 4b). The difference in $\delta^{13}\text{C}$ values between roots and SOM was not large enough to reliably partition respiration into old and new components for 1997 and 1998. The proportion of new C respired was highest from the NC treatment and lowest from the EC and AC treatments (Fig. 4a). The cumulative new C respired was highest from the NC treatment, and not different between EC and AC treatments, despite the presence of significantly more mineralizable C in the EC treatment (Table 1; Fig. 4b). New C made up 30% of the cumulative mineralized C in EC and AC treatments and 50% in the NC treatment (Table 1). This finding implies that decom-

position of new C inputs was not altered in EC compared to AC treatments, but that old (unlabeled) C was preferentially decomposed in the chambered treatments (70%) compared to the NC treatment (50%).

The $\delta^{13}\text{C}$ of new residue inputs from plants were always lower than other C pools, especially in the EC treatment, which received fossil fuel CO_2 (Table 4). The $\delta^{13}\text{C}$ values of all C pools were lowest in the EC treatment (except total SOC in 1997), demonstrating the incorporation of the fossil fuel ^{13}C label into all soil C pools. The $\delta^{13}\text{C}$ values of C pools in our experiments increased with age or MRT of the pool for all the treatments (except for AC slow pool in 1997). Active pool $\delta^{13}\text{C}$ was generally lower than slow pool $\delta^{13}\text{C}$, and total SOC had the highest $\delta^{13}\text{C}$ values. The increase in $\delta^{13}\text{C}$ values from new to active to slow to total SOC pools was strongest in EC treatment, but still noticeable in AC and NC treatments (Table 4). $\delta^{13}\text{C}$ of respired CO_2 was always lower than that of total SOC.

4. Discussion

The results of our incubation study show larger active and slow C pool sizes under elevated CO_2 . For active pool C in topsoil, the ratio of EC/AC increased systematically with time, suggesting that the active C pool was increasing under elevated CO_2 (Table 2). The topsoil slow pool C was larger in EC compared to AC in all three experiments, but the ratio of EC/AC did not change systematically for slow pool C. The incubation method appears to be a more sensitive method for detecting changes in soil C than measuring total SOC inventories. Turnover rates of active and slow pool C estimated in the laboratory experiments were not consistently affected by the first 3 years of elevated CO_2 treatments, suggesting that pool sizes may continue to increase under elevated CO_2 . Dynamic changes in $\delta^{13}\text{C}$ of respired CO_2 over the course of the incubation experiments are best explained by shifts in the proportion of active, slow, and (possibly) resistant C pools utilized by microbes.

4.1. Changes in SOM pool sizes

If elevated CO_2 stimulates C allocation belowground (e.g., Pendall et al., 2004a), larger active pool sizes would be expected to show up soon after treatments started owing to increased root exudation, fine root turnover, and substrate availability for microbial growth. Our incubation approach showed that elevated CO_2 increased mineralizable C after 2 and 3 years in topsoils but not subsoils. Mineralizable C was lower in the 1997 experiment than in the other years (Table 1), possibly because primary productivity was lowest during this year (Morgan et al., 2004), and/or because soils were not packed into beakers and may have dried out somewhat during that experiment. The large active pool size in the 1998 experiment (Table 2) may be explained by high biomass production rates in 1998 compared to the other years (Morgan et al., 2004). The

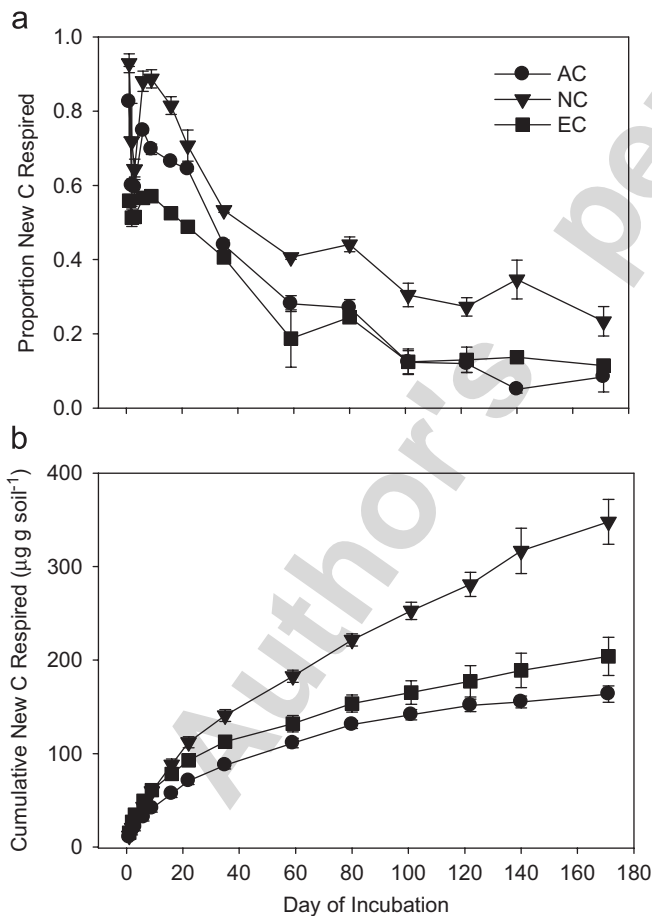


Fig. 4. (a) Proportion of new (^{13}C -labeled) C respired in 1999 incubation experiment based on isotope partitioning using Eq. (2) and (b) cumulative new C respired from topsoils of 1999 experiment. Abbreviations as in Fig. 1.

active pool C increased in EC relative to AC treatments systematically over the first 3 years of exposure to elevated CO₂ in topsoils and to a lesser degree in subsoils. These results are consistent with independent results from the same OTC study showing that rhizodeposition rates doubled (Pendall et al., 2004b) and root production increased under elevated CO₂ (Milchunas et al., 2005). Roots from elevated CO₂ treatments had higher fractions of soluble compounds (Milchunas et al., 2005), which is consistent with a larger active soil C pool. A similar incubation experiment showed that active pool C increased under elevated CO₂ in ponderosa pine (Haile-Mariam et al., 2000). We also detected slight increases in topsoil slow pool C under elevated CO₂, in contrast to the findings of Haile-Mariam et al. (2000).

A chamber effect was apparent in the 1998 and 1999 experiments in the form of substantially reduced slow and total C pool sizes in topsoil of the chambered treatments compared to the NC treatment (Table 2). The loss of C in topsoil of chambered treatments was partly offset by increased C pools in subsoil. Inside the chambers, soil temperatures were about 1.25 °C higher and soil moisture was slightly lower than outside (Morgan et al., 2004). These differences may have contributed to altered SOM dynamics, and emphasize the need for experimental designs that minimize changes in microclimate. Nonetheless, CO₂ treatment effects could still be examined in the AC and EC plots.

Active pool C ranged from 0.5% to 5% of the total C, and was similar to the proportions found in other studies (Collins et al., 2000; Haile-Mariam et al., 2000; Paul et al., 2001). Active pool sizes of 1% or lower may be attributable to the sandy soil texture and dry conditions at our study site, which may reduce microbial activity (Franzluebbbers et al., 2001) and reduce stabilization of the SOM. We used air-dried rather than field-moist soils in the incubations; this practice was not found to affect pool sizes in corn-belt soils (Collins et al., 2000).

4.2. Kinetics of SOM transformations

MRT is defined as the inverse of the turnover rate. Active pool MRTs of between 1 and 5 days found in all treatments of our experiment can be compared with MRTs of glucose (1 day) and fungal cytoplasm and cell wall material (10 days) (Paul and Clark, 1996). MRTs of slow pool C ranged from 2 to 6 years, which is shorter than the range of 15–45 years found in a recent literature survey (Paul et al., 2006). Several factors may have contributed to our relatively fast turnover rates. First, our incubation conditions were warmer and moister than average field conditions. We decided not to use an arbitrary Q_{10} value to adjust turnover rates to field temperatures. Comparison with field conditions may be facilitated by retaining soils at field moisture content, but field moist conditions introduce variability. Second, we rewetted air-dried soils, which likely did not affect the active pool size (Franzluebbbers et al.,

2001), but may have stimulated active pool turnover rates by 2–5 times (Collins et al., 2000). Third, our air-dried soils were also root-free, which may have affected pool sizes and turnover rates. Removing roots from soil before incubation has been shown to decrease microbial C and dissolved organic C compared to intact cores, but no effect was noted on basal respiration rate or Q_{10} values (Fang et al., 2005). Townsend et al. (1997) found that the incubation estimates of slow pool turnover rates were faster than the estimates from modeling or rough field estimates. Clearly the conditions of individual incubation experiments must be considered when comparing across experiments.

Increased C:N ratio of plant litter produced under elevated CO₂ fueled speculation that turnover rates of litter and SOM would decline and soil C storage should increase (Norby et al., 2001). However, leaf litter decomposition rates are often not altered by growth under elevated CO₂ conditions (e.g., Hirschel et al., 1997; Norby et al. 2001; De Graaff et al., 2004). No CO₂ treatment effects were observed on SOM turnover rates determined from long-term incubations on an elevated CO₂-OTC experiment with ponderosa pine (Haile-Mariam et al., 2000). Likewise, our incubation experiments indicated no change in active and slow pool turnover rates in soils exposed to elevated CO₂. However, these laboratory results are in conflict with our *in situ* results of increased decomposition of older SOM demonstrated by isotope partitioning of the soil respiration flux (Pendall et al., 2003). Possibly, decomposition of slow pool C was stimulated by rhizosphere priming (e.g., Dijkstra et al., 2006) in the field but not in the laboratory experiment.

It has been suggested that elevated CO₂ stimulates the rate of active pool C cycling but does not alter slow pool dynamics (Hungate et al., 1997). In 1999 sufficient isotopic labeling allowed us to compare mineralization of new C inputs across the experiment and demonstrated that new C turnover was not enhanced by elevated CO₂. This approach confirms that new C inputs under elevated CO₂ are not simply lost to mineralization. Further research incorporating isotope tracers, incubations and longer-term field experiments are needed to reconcile whether elevated CO₂ alters kinetics of slow pool C.

4.3. Dynamics of ¹³C during decomposition

A shift in $\delta^{13}\text{C}$ of respired CO₂ from lower to higher values during soil and plant decomposition experiments has been reported before (e.g., Schweitzer et al., 1999; Plante and McGill, 2002; Crow et al., 2006). However, we are not aware of reports of decreasing $\delta^{13}\text{C}$ values as labile C pools are used up, as we observed near the end of all three long-term incubation experiments. The isotopic changes we observed in EC soils were of a greater magnitude than reported in the literature because of the isotopic label those soils received. Several processes can potentially explain these isotopic dynamics.

In topsoils, the low $\delta^{13}\text{C}$ values observed during the active pool phase were similar to plant residue inputs; $\delta^{13}\text{C}$ increased during the slow pool phase toward the value of bulk SOM (Figs. 3a, c, and e). A likely explanation for this pattern is that microbes primarily utilized fresh residues during the active phase and utilized an increasing proportion of ^{13}C -enriched, older SOM as the incubation proceeded (Wedin et al., 1995; Townsend et al., 1997; Ehleringer et al., 2000). Indeed, we assume that microbes utilize changing proportions of old and new substrates in applying the $\delta^{13}\text{C}$ values to partitioning the two-pool model. Slow-pool phase $\delta^{13}\text{C}$ values were especially stable in the topsoil of the 1999 experiment. The steady decline in AC in the 1997 experiment to values more similar to older SOM probably resulted from a lower initial amount of active pool C. Variations in $\delta^{13}\text{C}$ values between days 130 and 150 in the 1998 experiment may have resulted from moving the samples between incubation cabinets.

The final decomposition phase of the 1998 and 1999 experiments was characterized by decreasing $\delta^{13}\text{C}$ values and may represent utilization of isotopically depleted lignin or resistant pool C when microbes were strongly C limited. The $\delta^{13}\text{C}$ value of lignin was found to be 2–6‰ lower than bulk plant tissue in a variety of plant tissue samples (Benner et al., 1987), and about 3.5‰ lower than bulk plant tissue in C3 and C4 grasses (Wedin et al., 1995). Topsoil NHC residue measured at the end of the OTC experiment had $\delta^{13}\text{C}$ values of –22.5‰ for AC and NC treatments and –23.5‰ for the EC treatment (E. Pendall, unpublished data), and $\delta^{13}\text{C}$ values of respired CO_2 decreased toward these low values at the end of each experiment. Evidence for microbial lignin utilization has been previously suggested. For example, in deciduous and coniferous forest soils, $\delta^{13}\text{C}$ of CO_2 respired from heavy density fractions ($>1.6\text{ g ml}^{-1}$) was sometimes lower than that from light fractions, as would be expected if microbes were utilizing isotopically depleted lignin residues (Crow et al., 2006).

In subsoils, initially high $\delta^{13}\text{C}$ of respired CO_2 during the first 3–5 days may have been caused by dissolution of trace quantities of calcium carbonate found below 30 or 40-cm depth in these soils (E. Paul, personal communication). After the first few days, the $\delta^{13}\text{C}$ values of respired CO_2 decreased steadily (Figs. 3b, d, and f). No steady-state phase appeared, and therefore we did not attempt to characterize $\delta^{13}\text{C}_a$ or $\delta^{13}\text{C}_s$ for subsoils. Microbes may have utilized increasing proportions of lignin or resistant pool C after about 130 days in the 1998 and 1999 experiments. $\delta^{13}\text{C}$ of subsoil NHC residue was –21.6‰ for AC and NC treatments and –22.3‰ for the EC treatment at the end of the OTC experiment, and increased reliance on this resistant C may explain decreasing $\delta^{13}\text{C}$ values of respired CO_2 with duration of incubation. Respired CO_2 was always more ^{13}C -enriched in subsoils than in topsoils. Cleveland et al. (2004) suggested that ^{13}C -enriched non-humic dissolved organic C is more likely

to be found lower in soil profiles than the ^{13}C -depleted humic fraction.

Microbial utilization of SOM pools with contrasting isotopic composition, and not fractionation during decomposition, probably explains most of the isotopic dynamics observed during our experiments. These patterns are similar to shifts from depleted to enriched respired CO_2 observed in other incubation experiments (e.g., Crow et al., 2006). Slow pool C may become enriched in ^{13}C compared to recent inputs or active pool C as ongoing catabolic carboxylation reactions incorporate ^{13}C -enriched soil CO_2 (Ehleringer et al., 2000). Resistant pool C may be depleted in ^{13}C relative to slow pool C because fresh lignin is included in the acid hydrolysis residue (Paul et al., 2006), and lignin is ^{13}C -depleted relative to average organic matter (Benner et al., 1987). Decreasing atmospheric $\delta^{13}\text{C}$ values due to fossil fuel burning (leading to lower values in SOM <100 yr in age), may explain some of the isotopic changes during decomposition. These isotopic dynamics should be accounted for in order to perform accurate partitioning estimates between rhizosphere respiration and SOM decomposition (Pendall et al., 2001, 2003; Rochette and Flanagan, 1997), when interpreting $\delta^{13}\text{C}$ values of ecosystem respiration (Pataki et al., 2002), and for inverse modeling studies (Ciais et al., 1995). A more mechanistic understanding of isotope dynamics during SOM decomposition will facilitate efforts to quantify changes in the global C cycle.

5. Conclusions

Our estimates of soil C pools and turnover rates using total C and C isotopic composition data allow examination of the response of this shortgrass steppe ecosystem to elevated atmospheric CO_2 . The results indicate that the active and slow C pools increased in the surface soil under elevated CO_2 and that there was not a simultaneous increase in turnover rates. These results suggest that soil C storage may increase in semi-arid grasslands under elevated CO_2 . Further studies employing stable isotope tracers and various SOM partitioning methods should be undertaken to examine the processes controlling these soil C dynamics. In particular, more long-term (>3 yr) field experiments investigating kinetics of pools with decadal residence times and depth-dependent responses of SOM to global change drivers are needed.

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