

**A dual isotope approach to isolate carbon pools**

M. S. Torn et al.

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# A dual isotope approach to isolate carbon pools of different turnover times

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## Abstract

Soils are globally significant sources and sinks of atmospheric CO<sub>2</sub>. Increasing the resolution of soil carbon turnover estimates is important for predicting the response of soil carbon cycling to environmental change. We show that soil carbon turnover times can be more finely resolved using a dual isotope label like the one provided by elevated CO<sub>2</sub> experiments that use fossil CO<sub>2</sub>. We modeled each physical soil fraction as two pools with different turnover times, using the atmospheric <sup>14</sup>C bomb spike in combination with the label in <sup>14</sup>C and <sup>13</sup>C provided by an elevated CO<sub>2</sub> experiment in a California annual grassland.

In sandstone and serpentine soils, the light-fraction carbon was 20–40 % fast cycling with 2–10 yr turnover and 60–80 % slow cycling with turnover slower than 100 yr. This validates model treatment of the light fraction as active and intermediate cycling carbon. The dense, mineral-associated fraction also had a very dynamic component, consisting of 5–10 % fast cycling carbon and 90–95 % very slow cycling carbon. Similarly, half the microbial biomass carbon in the sandstone soil was more than five years old, and 40 % of the carbon respired by microbes had been fixed more than five years ago.

Resolving each density fraction into two pools revealed that only a small component of total soil carbon is responsible for most CO<sub>2</sub> efflux from these soils. In the sandstone soil, 8–11 % of soil carbon contributes more than 85 % of the annual CO<sub>2</sub> efflux. The fact that soil physical fractions, designed to isolate organic material of roughly homogeneous physico-chemical state, contain material of dramatically different turnover times is consistent with recent observations of rapid isotope incorporation into seemingly stable fractions, and with emerging evidence for hot spots of decomposition within the soil matrix. Predictions of soil response using a turnover time estimated with the assumption of a single pool per fraction would greatly overestimate near-term response to changes in productivity or decomposition rates. Therefore, these results suggest more

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rapid, but more limited, potential for change in soil carbon storage due to environmental change than has been assumed by more simple mass-balance calculations.

## 1 Introduction

Soils are globally significant carbon (C) sources and sinks (Amundson, 2001). Soil organic matter (SOM) is a complex mixture of substances having turnover times ranging from days to millennia. Although models with a limited number of pools or SOM categories do a good job of predicting steady-state carbon stocks, they may not be adequate for many purposes (Davidson et al., 2000; Derrien and Amelung, 2011). Increasing the resolution of soil C turnover estimates is important for understanding biogeochemical cycling of carbon, nitrogen, and other SOM-related elements, as well as predicting the response to environmental change (Schimel et al., 1994; Trumbore, 2000; Torn et al., 2009).

Experiments simulating elevated atmospheric CO<sub>2</sub> levels are important for understanding ecosystem responses to rising atmospheric CO<sub>2</sub> concentrations (Norby and Zak, 2011). While elevated CO<sub>2</sub> did not significantly alter surface soil C stocks in annual grasslands (Hungate et al., 1996), there is evidence that it increases C partitioning to rapidly cycling pools below ground (Hungate et al., 1997). Moreover, under elevated CO<sub>2</sub>, soil microorganisms consume less older soil organic carbon (SOC) relative to easily degradable rhizodeposits from increased root biomass (Cardon et al., 2001). At a process level, elevated CO<sub>2</sub> can cause a 20–80% increase in soil enzyme activity without a change in microbial biomass (Kandeler et al., 2006). These experiments suggest that elevated CO<sub>2</sub> concentrations may cause a greater increase in rates of carbon cycling than in carbon storage (Norby and Zak, 2011), at least in annual grasslands (Hungate et al., 1997). Observations of this kind need to be rigorously evaluated before they can be generalized and incorporated into ecosystem models. This, in turn requires procedures that give reliable estimates of soil carbon turnover.

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Some studies using isotopic or stock/flow methods to determine soil carbon turnover times have used the simplifying assumption that SOM within one soil horizon represents a homogeneous, well-mixed pool with respect to decomposition (e.g., Raich and Schlesinger, 1992; Giardina and Ryan, 2000). However, organic materials in the soil have different decomposition kinetics, depending on chemistry, location, and physico-chemical conditions (Schmidt et al., 2011). For example, the proportion of SOM cycling with millennial turnover time can vary from zero to over 50 %, depending on mineral type, clay content, and soil depth (Torn et al., 1997; Masiello et al., 2004). This variation has a large effect on the calculated turnover times when soil-carbon fractions cycling at different rates are averaged together (Trumbore, 2000). Although it is widely recognized that assumptions of homogeneity in decomposition kinetics are too simplistic to adequately model and predict soil carbon turnover (Trumbore, 1993; Davidson et al., 2000; Torn et al., 2009; Derrien and Amelung, 2011), in practice, it is difficult to adequately describe and model turnover of an infinite number of individual organic compounds. While there are models representing decomposing organic matter as a continuum of differentially behaving compounds (Ågren and Bosatta, 1987), more commonly ecosystem models operationally divide SOM into a limited number of pools. For example, CENTURY model uses three pools designated active (annual turnover), intermediate (decadal-century) and passive (century-millennia) pools (Parton et al., 1987; Schimel et al., 1994).

Physical fractionation procedures separate SOM into pools of similar physico-chemical state based on aggregate, particle size, or density (Christensen, 2001; von Lützow et al., 2007). Density fractionation is applied to separate mineral-associated organic matter from free particulate organic matter (Sollins et al., 1983; Golchin et al., 1994; Crow et al., 2007; Moni et al., 2012). Organic matter in the light fraction (LF) decomposes more quickly than in the dense fraction (DF) (Sollins et al., 1984; Swanston et al., 2002; Mañín-Spiotta et al., 2008). In three-pool models, mineral-protected C (typically parameterized with texture) is part of the passive fraction and the LF is treated as a mixture of active and intermediate-cycling C (Trumbore, 2000). However, studies

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using isotopic composition to estimate turnover time of the LF (or free LF where occluded material is analyzed separately) often interpret the fraction data with a one-pool model (i.e., as all intermediate cycling). Theoretical considerations together with experimental evidence (Trumbore and Zheng, 1996; Swanston et al., 2005; Sollins et al., 2009) suggest that the DF is also too heterogeneous to be treated as one pool. In fact, the DF has shown rapid incorporation of both  $^{14}\text{C}$  (Swanston et al., 2005) and  $^{13}\text{C}$  (Bird et al., 2008), indicating that the dense fraction comprises a fast-cycling component as well as older carbon. Sequential density fractionation (e.g., Sollins et al., 2006, 2009) has isolated different pools with old radiocarbon ages, but has not explained or revealed the rapid incorporation of new organic inputs. The same is true for “free light” and “occluded light” fractions, which can have very different  $^{14}\text{C}$  contents, though none cycling on annual timescales (Golchin et al., 1994; Rasmussen et al., 2005; McFarlane et al., 2013). We are not aware of a method to physically separate the light fraction into active and slow cycling components, or to isolate the fast cycling part of dense, mineral-associated C. Therefore, we tested a dual isotopic labeling approach to achieve a functional separation of soil fractions into additional turnover time pools.

Soil microbes are the principal decomposers in soil, influencing nutrient cycling, soil carbon stocks, and plant productivity. In conventional three-pool soil carbon models, microbial biomass is treated as part of the active or fast pool, which makes up less than 5 % of the soil organic carbon and has turnover times in the range of years or less (Parton et al., 1987; Jenkinson, 1990). The isotopic composition of microbial biomass matches that of the substrates they utilize plus a discrimination factor (Dijkstra et al., 2006), and is useful for investigating microbial metabolism and substrate utilization (Kramer and Gleixner, 2006; Garnett et al., 2011). We tested the hypotheses that microbial biomass mainly assimilates recently fixed carbon. To do so, we employed the  $^{13}\text{C}$  and  $^{14}\text{C}$  label from an elevated  $\text{CO}_2$  experiment to quantify the amount of microbial biomass that was older than five years, which also provided a measure of the decomposition of older organic matter and its influence on heterotrophic respiration.

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The objective of this study was to contribute to understanding of SOM turnover dynamics by characterizing the heterogeneity of soil carbon turnover characteristics. We use the isotopic label ( $^{14}\text{C}$  and  $^{13}\text{C}$ ) from fossil fuel-derived  $\text{CO}_2$  used in an elevated  $\text{CO}_2$  experiment in an annual grassland, combined with the  $^{14}\text{C}$  bomb spike, to investigate soil carbon turnover and pool structure. We investigate two physical soil fractions, the mineral-associated, dense fraction and the light fraction, and determine turnover times when modeled as two pools in each fraction. Finally, we apply the dual isotopic label to characterize the extent to which microbial biomass comprises older versus recently assimilated carbon, and to estimate the fraction of heterotrophic respiration derived from decomposition of organic material fixed pre-experiment.

## 2 Materials and methods

### 2.1 The dual isotope and inverse label approach

Our approach takes advantage of the fact that the organic carbon entering the soil in the elevated  $\text{CO}_2$  experiment at Jasper Ridge, California from January 1992 to June 1997 (Field et al., 1996) was inversely labeled with two isotopes,  $^{13}\text{C}$  and  $^{14}\text{C}$ . In the case of  $^{14}\text{C}$ , this inverse labeling (dilution with  $^{14}\text{C}$ -free carbon) is superimposed on the enrichment  $^{14}\text{C}$  labeling of ecosystems caused by nuclear weapons testing (Fig. 1). This anthropogenic  $^{14}\text{C}$  signature or “bomb spike” has been decreasing since it peaked in the early 1960s.

Most elevated  $\text{CO}_2$  experiments apply  $\text{CO}_2$  produced from fossil fuels. Because fossil fuels are  $^{14}\text{C}$ -dead and depleted in  $^{13}\text{C}$  relative to ambient atmosphere, the assimilation of fossil fuel-derived  $\text{CO}_2$  results in depletion of both  $^{13}\text{C}$  and  $^{14}\text{C}$  in plant tissues. For example, at Jasper Ridge the fossil fuel versus atmosphere values of  $\text{CO}_2$  were  $\delta^{13}\text{C} = -35\text{‰}$  vs.  $-8\text{‰}$ , and  $\delta^{14}\text{C} = -1000\text{‰}$  vs.  $+100\text{‰}$  in 1996. We call this phenomenon “inverse labeling” because the procedure labels plant materials not by enriching, but by depleting the  $^{13}\text{C}/^{12}\text{C}$  or  $^{14}\text{C}/^{12}\text{C}$  ratio of plant tissue or a

specific compound. The inversely labeled (i.e., isotopically depleted) plant C is transferred through plant mortality and exudates to the soil, where it gradually replaces “old” C (C fixed pre-experiment) that is cycling out of the soil. The degree to which soil C or a particular soil C fraction has incorporated the depleted plant signal indicates the proportion of soil C that has turned over during the course of the experiment (Fig. 1).

We estimate turnover times based on a linear donor-controlled model of soil C cycling (Trumbore, 1993; Torn et al., 2009), meaning that the amount of C decomposed is the product of the C stock (C), a decomposition rate constant ( $k$ ), and the time interval ( $\Delta t$ ). The turnover time ( $\tau$ ) is the reciprocal of the decomposition rate (i.e.,  $\tau = 1/k$ ).

The Jasper Ridge grassland is dominated by annual plant species, allowing us to make an assumption that simplifies the modeling task, namely that all plant C inputs to soil in a given year have the isotopic content of that year’s net primary productivity (NPP). This assumption is weakest in the subsoil, where some C inputs may have been leached from surface to subsoil on occasions when precipitation exceeded field capacity. We also assumed that leaching and erosion losses from these soils are negligible, so that all carbon losses can be attributed to organic matter decomposition (alternatively, one could restate the turnover time as pertaining to all loss pathways rather than just decomposition).

The change in soil C stock between one year and the next is the difference between the inputs and outputs over that year. Thus, the amount of organic carbon present in the soil-depth interval at a given time, as a function of the rate of plant inputs and the turnover time for decomposition is:

$$C(t) = C_{t-1} + I_{t-1} - \left( \frac{C_{t-1}}{\tau} \right) \quad (1)$$

where  $C(t)$  = soil C stock at year  $t$  ( $\text{kg m}^{-2}$ );  $C_{t-1}$  = soil C stock at the year before year  $t$ ;  $t$  = annual time step ( $y$ );  $I$  = plant C inputs ( $\text{kg m}^{-2} \text{yr}^{-1}$ ); and  $\tau$  = turnover time of soil C( $y$ ).

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An analogous relationship holds for isotopic content: the change in soil C isotopic ratio between one year and the next depends on the difference between the isotopic content of the annual inputs and outputs. The amount of  $^{13}\text{C}$  can be expressed by multiplying the  $\delta^{13}\text{C}$  value by total C content:

$$\delta^{13}\text{C}_{\text{soil}}(t) = \frac{(\delta^{13}\text{C}_{\text{soil},t-1} \cdot C) + (\delta^{13}\text{C}_{\text{plant},t} \cdot I_t) - \left(\frac{\delta^{13}\text{C}_{\text{soil},t-1} \cdot C}{\tau}\right)}{C} \quad (2)$$

where  $C$  = amount of total soil carbon at steady state ( $\text{kg m}^{-2}$ );  $t$  = time step ( $y$ );  $\delta^{13}\text{C}_{\text{soil}}(t) = ^{13}\text{C}$  content of soil at time  $t$  (‰);  $\delta^{13}\text{C}_{\text{plant}}(t) = ^{13}\text{C}$  content of plant input to soil at time  $t$  (‰);  $I$  = input rate of plant C ( $\text{kg m}^{-2} \text{yr}^{-1}$ ); and  $\tau$  = turnover time of soil organic carbon ( $y$ ).

In the case of  $^{14}\text{C}$ , a second loss pathway, radioactive decay, is added to the existing decomposition loss term.

$$\Delta^{14}\text{C}_{\text{soil}}(t) = \frac{(\Delta^{14}\text{C}_{\text{soil},t-1} \cdot C) + (\Delta^{14}\text{C}_{\text{plant},t} \cdot I_t) - \left(\left(\frac{1}{\tau} + \lambda\right) \cdot (\Delta^{14}\text{C}_{\text{soil},t-1} \cdot C)\right)}{C} \quad (3)$$

where  $\delta^{14}\text{C}_{\text{soil}}(t) = ^{14}\text{C}$  content of soil at time  $t$  (‰);  $\delta^{14}\text{C}_{\text{soil}}(t-1) = ^{14}\text{C}$  content of soil at time  $t-1$  (‰);  $t$  = time step ( $y$ );  $\delta^{14}\text{C}_{\text{plant}}(t) = ^{14}\text{C}$  content of plant inputs at time  $t$  (‰); and  $\lambda$  = radioactive decay constant for  $^{14}\text{C}$  ( $0.000121 \text{ yr}^{-1}$ ).

Under steady-state conditions, i.e., when annual plant inputs equal annual decomposition losses, there is no change in carbon stock from year to year. In other words,  $C(t) = C(t-1)$ . In that case, that term can be substituted into Eq. (1), yielding the following:

$$I = \frac{C_t}{\tau} = \frac{C_{t-1}}{\tau} = \frac{C}{\tau} \quad (4)$$

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with the steady-state assumption for carbon stocks and constant rate of plant C inputs, the input term,  $I$ , can be replaced with  $C/\tau$ , greatly simplifying the equations used to solve for isotopic values over time.

## 2.2 Study site and soil sampling

5 Our study was located in annual grasslands at Jasper Ridge Biological Preserve at Stanford University, California, USA (37° 24' N, 122° 14' W) in central coastal California. Two types of grassland, differing in plant species composition, productivity, and nutrient limitation, occur adjacent to each other on sandstone- and serpentine-derived soils. The moderately productive sandstone grasslands have well-developed soils and  
10 are dominated by exotic European annual grasses. The serpentine grasslands have shallow, poorly developed soils and lower productivity under a mix of exotic annuals and native forbs and grasses. In the Jasper Ridge elevated CO<sub>2</sub> experiment (Field et al., 1996), 0.3 m<sup>2</sup> plots in open-top chambers were exposed to either ambient ( $n = 10$ ) or elevated (ambient + 350 ppm,  $n = 10$ ) CO<sub>2</sub> from January 1992 to June 1997.

15 Soil cores were collected in April 1997 from sandstone (0–15 and 15–30 cm) and serpentine (0–15 cm) field plots, air-dried, and dry-sieved (2 mm) for rocks and roots. Rock fragment content, defined as the weight fraction of mineral material that did not pass through a 2 mm sieve, was determined to be 6 % weight for the sandstone soil and 41 % weight for the serpentine soil. We sampled sandstone soil bulk density at  
20 0–15 and 15–30 cm (0.94 and 1.28 g cm<sup>-3</sup>, respectively) depths by taking volumetric samples from the side of a soil pit near mid point of the respective depth interval with a Soil Moisture brand bulk density sampler. Samples were oven-dried at 105 °C before weighing. We also used bulk-density data from Hungate et al. (1996) who sampled  
25 each plot once with a known-volume corer from the soil surface to obtain bulk density samples in the 0–15 cm depth (0.97 g cm<sup>-3</sup>) in the serpentine grassland.

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## 2.3 Density fractionation and isotopic analysis

We used density separation to isolate light and dense (mineral-associated) soil carbon pools. Ten grams soil subsamples were suspended in 50 mL of sodium polytungstate (Geoliquids, Inc.) solution of  $2.0 \text{ g mL}^{-1}$  (sandstone) or  $1.8 \text{ g mL}^{-1}$  (serpentine) and centrifuged  $2 \times 30 \text{ min}$  at 2500 rpm (1500 g) in pre-weighed centrifuge tubes. After testing, the less dense solution was used for serpentine soil, because at  $2.0 \text{ g mL}^{-1}$ , some small serpentine mineral material was visible in the light fraction. Density was adjusted until the two soils presented similar (and very low) amounts of visible mineral material in the light fractions. Between centrifuge runs, floating (light fraction) material was pipetted onto pre-weighed nylon membrane filters in Nalgene filtration units, and the pellet was re-suspended. Filters were rinsed with 200 mL deionized water, oven dried 24 h at  $80^\circ\text{C}$ , and reweighed. Dense fraction material remained in the pellet, which was rinsed in 50 mL deionized water by repeated centrifuging ( $4 \times 5 \text{ min}$ , 1500 g) then oven-dried 72 h at  $80^\circ\text{C}$  in the centrifuge tubes and weighed. Light fractions were ground by hand; dense fractions and whole (unfractionated) soil samples were ground with steel rods in capped plastic vials on a roller bar grinder.

Subsamples of sieved soil were weighed into tin capsules and combusted in a Carlo-Erba C : N : S analyzer coupled to a Mass Spectrometer for %C and  $\delta^{13}\text{C}$  values. The C and N concentration was also measured by elemental analyzer at University of California, Berkeley.

Radiocarbon content was determined for 12 samples, each a composite of the 10 replicate plots for each soil (sandstone and serpentine), depth (two depths for the sandstone soil and one depth in the shallow serpentine soil), treatment (control and elevated  $\text{CO}_2$ ), and fraction (light and dense) combination. The low variation in soil  $\delta^{13}\text{C}$  among the treatment replicates showed that the amount of C from elevated- $\text{CO}_2$  inputs in each SOM fraction was relatively uniform. The graphite targets were prepared by oxidizing SOM to  $\text{CO}_2$  by sealed tube combustion with cupric oxide followed by reduction of the cryogenically purified  $\text{CO}_2$  to graphite by hydrogen or zinc reduction

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(Vogel, 1992). Samples were analyzed by accelerator mass spectrometry at Lawrence Livermore National Laboratory. Analytical precision was reported to be  $\pm 4\text{--}8\%$ . The  $\delta^{14}\text{C}$  unit is normalized such that for the pre-1950 atmosphere,  $\delta^{14}\text{C} = 0\%$  (Stuiver and Polach, 1977). Positive values of  $\delta^{14}\text{C}$  indicate the presence of bomb-produced  $^{14}\text{C}$ , and samples with no  $^{14}\text{C}$  have  $\delta^{14}\text{C} = -1000\%$  (half-life = 5730 yr). The calculation of fraction modern or  $\Delta$  from the sample activity uses sample  $^{13}\text{C}$  to correct for mass-dependent isotope fractionation. However, because the  $\delta^{13}\text{C}$  of the sample is not an appropriate measure of isotopic fractionation in elevated  $\text{CO}_2$ -treatments, sample  $^{14}\text{C}$  activity was corrected for isotopic fractionation following the procedure proposed by Torn and Southon (2001).

## 2.4 Modeling soil carbon turnover in density fractions

The turnover calculations presented here depend on three assumptions: (i) carbon cycling in the control plots is in steady state, (ii) atmospheric  $\delta^{13}\text{C}$  values are constant, and (iii) the elevated  $\text{CO}_2$  treatments only slightly altered decomposition rates so that both control and elevated  $\text{CO}_2$  treatments can be treated as having the same input rates and turnover times. (This assumption was tested by comparing the modeling results for control plots using bomb  $^{14}\text{C}$  versus elevated  $\text{CO}_2$  treatments using dual isotopes; Table 4.) The goal of the turnover modeling is to divide each SOM density fraction into two functional pools. The method is to come up at a turnover time or times such that the predicted soil fraction  $^{14}\text{C}$  and  $^{13}\text{C}$  most closely match the measured values. We iteratively changed the (i) relative proportions of fast vs. slow cycling C pools within a density fraction and (ii) the turnover times of the fast and slow cycling C pools of that density fraction until they matched the  $^{14}\text{C}$  observed in the control soil and the  $^{13}\text{C}$  and the  $^{14}\text{C}$  values measured in the soil from the elevated  $\text{CO}_2$  treatment. We modeled each density fraction independently.

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## 2.4.1 One-pool and two-pool models

To compare the results of turnover calculations that assume one or two pools per fraction, we estimated the turnover times of organic carbon in the density fractions using the same time-dependent, steady-state equations with either one pool or two pools. In both approaches, we assume each pool represents a well-mixed, homogeneous C pool where all organic compounds have equal probability of turnover (loss due to decomposition). For the one-pool model, each density fraction is a single pool. For the two-pool case, the model predicts the  $^{14}\text{C}$  and  $^{13}\text{C}$  value for each pool and the proportion,  $p$ , of C in each pool. Then the predicted fraction-total  $^{14}\text{C}$  and  $^{13}\text{C}$  was compared with the measured isotopic composition of the fraction, where the fraction-total isotopic value was calculated as the mass-weighted average of the two pools as shown here for  $^{13}\text{C}$ :

$$p_{\text{fast}} + p_{\text{slow}} = 1 \Rightarrow p_{\text{fast}} = 1 - p_{\text{slow}} \quad (5)$$

$$C_t \delta_t = C_{\text{slow}} \delta_{\text{slow}} + C_{\text{fast}} \delta_{\text{fast}} \Rightarrow \delta_t = \frac{C_{\text{slow}}}{C_t} \delta_{\text{slow}} + \frac{C_{\text{fast}}}{C_t} \delta_{\text{fast}}$$

$$\Rightarrow \delta_t = p_{\text{slow}} \delta_{\text{slow}} + p_{\text{fast}} \delta_{\text{fast}} \quad (6)$$

$$\delta_t = p_{\text{slow}} \delta_{\text{slow}} + (1 - p_{\text{slow}}) \delta_{\text{fast}} \quad (7)$$

where  $p$  = proportion of C in each pool;  $C$  = C stock;  $\delta$  =  $\delta^{13}\text{C}$  value of soil; and subscripts are  $t$  = total sample (LF or DF); fast = fast-cycling C pool; and slow = slow-cycling C pool.

## 2.4.2 Quantifying the proportion of $\text{CO}_2$ flux attributed to fast and slow pools

The total annual C flux ( $\text{kg m}^{-2} \text{yr}^{-1}$ ) was calculated as the sum of the flux from the fast pool of the light fraction, the flux from the slow pool of the light fraction, the flux from the fast pool of the dense fraction, and the flux from the slow pool of the dense fraction:

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$$\text{Flux} = \frac{\text{LF} \cdot \rho_{\text{fast,LF}}}{\tau_{\text{fast,LF}}} + \frac{\text{LF} \cdot \rho_{\text{slow,LF}}}{\tau_{\text{slow,LF}}} + \frac{\text{DF} \cdot \rho_{\text{fast,DF}}}{\tau_{\text{fast,DF}}} + \frac{\text{DF} \cdot \rho_{\text{slow,DF}}}{\tau_{\text{slow,DF}}} \quad (8)$$

where LF = light fraction ( $\text{kg m}^{-2}$ ), DF = dense fraction ( $\text{kg m}^{-2}$ ),  $\rho$  = proportion of C in each fraction with fast or slow cycling rate, and  $\tau$  is turnover time (years) of either fast or slow cycling C in either light or dense fraction.

The proportion of each density fraction in fast and slow pools was scaled by total soil C to estimate how much of the annual flux could be attributed to the amount of soil carbon that is turning over rapidly or slowly.

### 2.4.3 Estimating turnover times

For each density fraction, turnover time ( $\tau$ ) was determined such that the model prediction of  $\delta^{14}\text{C}$  most closely matched the measured value for the respective density fraction. The model was solved using Eq. (3):

The modeling started in the year 1900, with the assumption of steady-state carbon flows:

$$R = \frac{1/\tau}{(1/\tau) + \lambda} \quad (9)$$

where  $R$  = the fraction modern of radiocarbon (dimensionless). Yearly atmospheric radiocarbon values were based on the literature (Stuiver et al., 1998; Hua and Barbetti, 2004).

Turnover times were modified in an iterative process until the resulting  $\delta^{14}\text{C}$  value for the respective density fraction matched the observed value from Table 2. To facilitate comparison of the one- and two-pool model results, we calculated the flux-weighted turnover time for the soil with two pools,  $\tau_{\text{effective}}$ , where total soil  $\text{CO}_2$  flux from the

whole soil is  $C/\tau_{\text{effective}}$ . This flux-weighted  $\tau$  was calculated as

$$\tau_{\text{effective}} = \frac{1}{\left(\frac{f_{\text{fast}}}{\tau_{\text{fast}}}\right) + \left(\frac{f_{\text{slow}}}{\tau_{\text{slow}}}\right)} \quad (10)$$

where  $\tau_{\text{effective}}$  = flux weighted turnover time,  $\tau_{\text{fast}}$  = turnover time of fast pool,  $\tau_{\text{slow}}$  = turnover time of slow pool,  $f_{\text{fast}}$  = weight fraction of fast pool, and  $f_{\text{slow}}$  = weight fraction of slow pool.

Turnover calculations were based on the data, references, and calculation methods compiled in Table 1. The proportion of fossil fuel air,  $f(0.444)$ , in the chamber air was calculated based on the measured  $^{13}\text{C}$  values in the chamber air ( $-20\%$ ), ambient air ( $-8\%$ ), and tank air ( $-35\%$ ). The  $^{14}\text{C}$  content of  $\text{CO}_2$  in the chambers each year was estimated by mass balance, assuming 0.444 of the air was fossil fuel derived with  $\delta^{14}\text{C} = -1000\%$  and  $(1-0.444)$  was ambient air with  $\delta^{14}\text{C}$  value of the Northern Hemisphere in that year.

#### 2.4.4 New carbon in each soil fraction

The percentage of soil C derived from C fixed since the beginning of the experiment ( $\%C_{\text{new}}$ ) was calculated following van Kessel et al. (2000):

$$\%C_{\text{new}} = \left( \frac{\delta^{13}\text{C}_{\text{elevated-soil}} - \delta^{13}\text{C}_{\text{ambient-soil}}}{\delta^{13}\text{C}_{\text{elevated-plant}} - \delta^{13}\text{C}_{\text{ambient-soil}}} \right) \times 100 \quad (11)$$

where  $\delta^{13}\text{C}$  values for plant samples ( $\delta^{13}\text{C}_{\text{elevated-plant}}$ ) were taken from Jackson et al. (1994) and  $\delta^{13}\text{C}$  values for soil fraction samples ( $\delta^{13}\text{C}_{\text{elevated-soil}}$  and  $\delta^{13}\text{C}_{\text{ambient-soil}}$ ) were measured directly.

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## 2.5 Microbial biomass

The chloroform fumigation-incubation method (Jenkinson and Powlson, 1976) was used to measure microbial biomass and to generate microbial-derived CO<sub>2</sub> for isotopic analysis. Soil from 0–5 cm of sandstone grassland was collected in April 1996, and refrigerated until used, within five days of collection. This was nearly five growing seasons after the experiment began. The soil was sieved (2 mm) and macroscopic organic matter fragments like roots and leaves were removed. Aliquots of 15 g were moistened to 20 % gravimetric moisture with distilled water and either incubated for 10 days (control) or fumigated with chloroform and then incubated for 10 days (fumi-  
gated). Three replicates of each treatment were incubated, but one of the jars from the elevated CO<sub>2</sub> treatment leaked during incubation and one <sup>14</sup>C sample from the control treatment was lost on the vacuum line.

After incubation, the headspace gas was analyzed for CO<sub>2</sub>, <sup>13</sup>C, and <sup>14</sup>C content. Samples for δ<sup>13</sup>C were removed with a gas-tight syringe and injected directly into a GC-Finnegan mass spectrometer at Carnegie Institution for Science, Stanford. The <sup>14</sup>C samples were composited for analysis. For δ<sup>14</sup>C, 1.5 mg C was trapped by pumping headspace gas over a cryogenic trap. Samples were graphitized as above, and δ<sup>14</sup>C was measured by accelerator mass spectrometry at Lawrence Livermore National Laboratory. Headspace CO<sub>2</sub> concentration was converted to mass using the ideal gas law at *T* = 293 K and 1 atm pressure. Microbial biomass C<sub>M</sub> was calculated as the difference in C respired in fumigated (C<sub>F</sub>) and control (C<sub>C</sub>) incubations divided by extraction efficiency (Jenkinson and Powlson, 1976).

The control (unfumigated) incubation gives an estimate of the isotopic values for heterotrophic respiration, although we do not expect it to be exactly the same as field values, because soil processing likely makes older C more bio-available. We estimated the isotopic composition of microbial biomass with a two-member mixing model, using the same assumptions that are in the microbial biomass calculation, namely that the amount of fumigated CO<sub>2</sub> is equivalent to the amount of respired CO<sub>2</sub> in the control

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incubation plus CO<sub>2</sub> liberated from microbial biomass cells that were lysed by the chloroform.

$$C_F = C_C + C_M \quad (12)$$

$$C_F \delta_F = C_C \delta_C + C_M \delta_M \quad (13)$$

Equations (12) and (13) can be combined and simplified, yielding:

$$\delta_M = \frac{C_F \delta_F - C_C \delta_C}{C_F - C_C} \quad (14)$$

where C<sub>M</sub> = microbial biomass carbon, C<sub>F</sub> = C respired in fumigated incubations, C<sub>C</sub> = C respired in control incubations, δ<sub>M</sub> = isotopic value of biomass carbon, δ<sub>F</sub> = isotopic value of carbon respired in fumigated incubations, and δ<sub>C</sub> = isotopic value of carbon respired in control incubations.

We used the microbial biomass results in three ways: (1) to estimate the fraction of microbial biomass that was derived from pre-experiment (1992) photosynthesis; (2) to estimate the fraction of heterotrophic respiration that was derived from pre-experiment photosynthesis; and (3) to use the microbial biomass isotopic values to constrain a model of the SOM pools being decomposed. The first two calculations use a two-member mixing model, as shown above for soil C (Eq. 11).

## 3 Results

### 3.1 New carbon in SOM fractions

The fractionation procedure separated the soil into fractions with distinct C, N, and isotopic values (Table 2). Across soils and treatments, LF accounted for 12–29% of total soil carbon. The proportion of total horizon C in LF was twice as high in the topsoil (0–15 cm) as in the 15–30 cm depth in the sandstone soil. Serpentine topsoils had



a higher proportion of C in LF than did sandstone topsoils. In both sandstone and serpentine topsoils, there tended to be more LF organic material in the elevated CO<sub>2</sub> treatment than in the control treatment.

Compared to the DF, the LF was more C-rich, had a wider C/N ratio, and, in the elevated CO<sub>2</sub> plots, had incorporated more of the fossil fuel isotopic signature (Table 2). These results are consistent with the LF consisting of plant material in an early stage of decomposition that is on average cycling more quickly than is the DF. In complement, the low C concentration, narrow C/N ratio, and old isotopic signature all indicate that the DF comprised more degraded material that was stabilized in association with soil minerals.

The distinctive <sup>13</sup>C signature of the elevated CO<sub>2</sub> plant inputs allowed us to determine the fraction of soil organic carbon derived from C photosynthesized after the experiment began (January 1992), hereafter called *new C*. Based on the incorporation of <sup>13</sup>C, significantly more (by a factor of 3–5) new C had entered the LF than the DF (Table 4). The C in the LF was 21–36% new, while less than 7% was new in any of the DF samples. This is consistent with the LF having faster turnover or at least having a larger pool of fast-cycling C than did the DF. There was more new C in the 0–15 cm depth than deeper in the sandstone soil. Comparing surface horizons between soil types, there was more new C in the serpentine topsoil than in the sandstone topsoil.

### 3.2 Soil carbon turnover times

The turnover-time estimates for the one-pool and two-pool models were summarized in Tables 3 and 4, respectively. Three broad trends are shown by both one-pool and two-pool modeling approaches. First, the light fractions turn over more quickly than do the dense fractions. Second, ecosystem C residence times increased with depth for both light and dense fractions. Third, turnover time in the serpentine soil is slower than that in the sandstone soil, for each comparable density fraction and soil depth.

Using a one-pool model (Table 3), soil <sup>14</sup>C values in the control treatment yielded turnover times of DF (275–742 yr) roughly double those of LF (124–406 yr). In the

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elevated CO<sub>2</sub> treatment, soil <sup>13</sup>C values resulted in decadal turnover for LF (18–36 yr) and centennial turnover for DF (105–420 y), while soil <sup>14</sup>C values did not isolate a unique solution for turnover time of either fraction (low and high estimates were roughly 10 and 100–1000 yr for a given sample).

We also applied the two-pool model to the control and elevated CO<sub>2</sub> treatments, separately and in a scenario where we assume that both treatments have identical turnover times (Table 4). If the one-pool and two-pool approaches were equivalent, the flux-weighted  $\tau_{\text{effective}}$  by the two-pool model would be similar to the turnover time obtained by the one-pool model, and thus the difference between the one-pool  $\tau$  and  $\tau_{\text{effective}}$  provides a measure of how well the one-pool model captures the overall flux and response time of the soil fraction. The large difference between the one-pool (Table 3) and flux-weighted  $\tau$  (Table 4) suggests that the single pool model is missing important dynamics of the system. More importantly, only the two-pool model could fit all three isotopic data sets (control treatment <sup>14</sup>C and elevated CO<sub>2</sub> treatment <sup>14</sup>C and <sup>13</sup>C, Table 2). For both these reasons, we use the two-pool model from combined control and elevated CO<sub>2</sub> treatments in the following results.

The LF in the sandstone soil consisted of 23–30 % fast-cycling C with ~ 3 yr turnover time regardless of depth. The amount and residence time of this fast-cycling component did not depend on depth. The turnover time of the slow-cycling component of LF, however, was 4 times slower in the 15–30 cm increment than at the surface (654 vs. 150 yr). Thus, 70–77 % of the sandstone LF-carbon cycles very slowly and has a turnover time that is influenced by depth. Moreover, in the serpentine soil, a greater proportion of the LF (54 %) was fast cycling with a relatively slower turnover time (11 yr), and a smaller proportion of the LF (46 %) was slow cycling with a slower turnover time (867 yr) than in the sandstone soil. Therefore, the flux-weighted  $\tau$  of the serpentine soil was a little slower (21 yr) than that of the sandstone soil (9–14 yr).

The %C<sub>new</sub> for the serpentine soil (36 %) was less than the %C<sub>fast</sub> (54 %), mainly because with turnover time of 11 yr, not all the C in the fast-cycling pool had been turned over (replaced by new C) after 6 yr of experiment. These results validate a modeling

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approach of treating the LF as a combination of active and intermediate cycling C with turnover times that differ by an order of magnitude.

Although the mineral-associated DF is typically considered to comprise all slow-cycling C, the DF data better fit a model with one significantly faster-cycling pool. The DF of sandstone and serpentine soils was 7–9% fast cycling C with turnover times (3–16 yr) similar to those of the LF fast cycling pool (3–11 yr) from the same soil. In contrast, the more stable pool of the DF of sandstone soil cycled much more slowly than did its LF counterpart (304–881 vs. 150–654 yr for DF and LF, respectively), and more slowly with depth (150–304 vs. 654–881 yr for 0–15 and 15–30 cm, respectively).

### 3.3 Microbial biomass

The isotopic signatures of microbial biomass carbon and biomass respiration of the sandstone topsoil (0–5 cm) are given in Table 5. Further calculations using two-member mixing models showed that nearly half the microbial biomass C was more than five years old and approximately 40% of the carbon respired by microbes had been fixed more than 5 yr ago. The same results were obtained from soil samples collected in 1997 (data not shown).

## 4 Discussion

### 4.1 New carbon in SOM fractions

In this study, the percentage of each soil physical fraction that was fast cycling ( $\%C_{\text{fast}}$ ) was close to the value for the  $\%C_{\text{new}}$  in that fraction. Considering all 6 soil type/depth/fraction cases,  $\%C_{\text{new}}$  explained 88% of the variability in  $\%C_{\text{fast}}$  ( $R^2 = 0.88$ ,  $P < 0.01$ , Fig. 2). More generally, the two values ( $\%C_{\text{new}}$  and  $\%C_{\text{fast}}$ ; Table 4) will be almost equivalent if (1) the duration of the experiment is longer than the turnover time of the fast pool, so that enough of the fast pool has turned over to new C; and (2) the

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turnover time of the slow pool is much longer than the duration of the experiment, so that very little of the slow pool will have accumulated new C. Under these conditions, %C<sub>new</sub> could be used as an easy method for estimating the minimum value of % fast cycling, even in conditions where it is not possible or affordable to model two pools explicitly.

## 4.2 Turnover times

As discussed above, the two-pool model gave a better and more robust fit to the data than did the one-pool model. In the two-pool model, there is no significant difference between turnover-times in the control and elevated CO<sub>2</sub> treatments. Therefore, we chose the two-pool model constrained by both the control and elevated CO<sub>2</sub> treatments simultaneously as our “best case” for turnover time values in the discussion below.

For all three soil type/depth cases, the turnover time of the fast pool was nearly the same in the light and dense fractions, while turnover of the slow pool was 1.5–2 times slower in the DF than in the LF (Table 4). This may be because the fast-cycling pool has similar organic matter chemistry in light and dense fractions, while the slow-cycling pool has different organic matter chemistry or physico-chemical stabilization between the two physical fractions. Therefore, LF turns over faster than DF, two reasons—LF has more fast-cycling carbon than does DF, and the slow-cycling carbon in LF turns over faster than that in DF.

Additionally, we observe that the turnover time of the fast-cycling pool (in LF or DF) does not change substantially with depth, while the turnover time of the slow-cycling pool increases 3–4 times when going from surface (0–15 cm) to the next horizon (15–30 cm) in sandstone grassland. One possible explanation is that, in contrast with the fast-cycling C, the turnover time of the slow-cycling material is influenced by physico-chemical stabilization that increases with depth. Thus, deep soil turns over slower than surface soil because of the much slower turnover of the stabilized material in deep soil than in surface soil, as well as the greater proportion of slow versus fast cycling carbon.

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Even though the plots shared the same climate and similar plant species composition, SOC turnover at the same depth was much slower in serpentine grassland than in sandstone grassland. There are many potentially relevant differences between the two soils: the serpentine grassland has lower productivity, lower plant biomass, different soil mineralogy and cation composition, and shallower soils such that the 0–15 cm is much closer to bedrock than for the sandstone grassland (Hungate et al., 1997). Although serpentine soils are uncommon, because they can be easily paired with other soils with the same climate and plant communities, they could provide an interesting natural experiment to explore the role of soil mineralogy on decomposition.

We showed that it is practical to partition soil physical fractions into multiple pools using a dual isotopic label, such as that provided by most elevated CO<sub>2</sub> experiments. A spreadsheet model for fitting two pools simultaneously is only slightly more complicated than that for fitting one pool to the data. An alternative method to partitioning the fractions is to use a single isotope over multiple time points, such as <sup>13</sup>C in soil from an elevated CO<sub>2</sub> experiment (van Kessel et al., 2000) or under C<sub>3</sub>–C<sub>4</sub> vegetation change (Balesdent et al., 1988). However, the use of <sup>14</sup>C allowed modeling of the control soil as well, which is not possible when only using <sup>13</sup>C.

The pattern in turnover times confirms earlier suggestions that fractionation of soil into light and dense fractions does not physically separate soil into homogeneous pools (Trumbore et al., 1989; Trumbore and Zheng, 1996). Even the mineral-associated dense fraction contains a range of turnover times (Trumbore, 2000), as sequential density fractionations have confirmed. But none of the sequential density fractionation studies (Sollins et al., 2006, 2009) or comparisons of free and occluded light fraction (Rasmussen et al., 2005; McFarlane et al., 2013) have isolated the fast cycling component of the dense or light fractions, respectively. A contribution of our work over previous studies is the mathematical isolation (constraint of) a small but very dynamic component of each density fraction.

The highly dynamic carbon pool has important biogeochemical implications. Having a small, very fast cycling pool of C means that a small fraction of total soil C is

responsible for most of the CO<sub>2</sub> efflux. Taking into account the C content and turnover time of each pool as well as the C content of the whole soil profile (Tables 2 and 4), in the sandstone soil, 8–11 % of soil C contributes more than 85 % of the annual CO<sub>2</sub> efflux. This finding shows that only about 10 % of the soil C stock could be lost rapidly, on the order of years, to environmental change such as elevated temperature or decreasing plant inputs. Conversely, about 90 % of the soil C is not dynamic on fast time scales. It has been established that studies using one pool per soil profile (Raich and Schlesinger, 1992; Giardina and Ryan, 2000) greatly overestimate the amount of moderately responsive C in the soil, while missing the small, most dynamic fraction (Trumbore, 2000). We find that even treating each physical fraction as a single pool – such as we and others have done in previous work (e.g., Rasmussen et al., 2005; Mikutta et al., 2006; Bird et al., 2008; McFarlane et al., 2013) – underestimates the near-term response of soil, and in the long term overestimates the size of the C pool that is involved in decadal scale fluxes. Isolating fractions, and modeling each fraction as a single pool, is still a large improvement over treating all soil C as one pool and is representative of the turnover time of most of the C in the sample, but it should be recognized that a small fraction of this mass accounts for most of the CO<sub>2</sub> flux and the dynamics.

Figure 3 compares the effect of assuming soil C comprises one versus two pools on predictions of the long-term response to changes in decay rate (Fig. 3a) or plant inputs (Fig. 3b). Both assumptions (one- vs. two-pool) result in the same stock at a new equilibrium, but with different response trajectories. The initial, sharp drop in soil carbon stock with doubling decay rate in the two-pool case (Fig. 3a) occurs because the fast pool approaches a new equilibrium stock within a few years. Subsequently, the slow pool has a very slow response time, and it takes about 300 yr for the whole fraction to reach the equilibrium stock. The single pool model has (by definition) an intermediate turnover time, and this allows for moderately rapid exponential decline in stock; it approaches the new equilibrium in about 20 yr (Fig. 3a). In other words, the one-pool model overestimates the amount of C that can be lost from the soil and transferred to

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atmosphere in the decades after an environmental perturbation, as well as overestimating the amount of C that can be sequestered in the soil in the next few decades if carbon inputs (as NPP) to soil were increased (Fig. 3b). This example also helps to illustrate how equating the mean,  $^{14}\text{C}$  derived *age* of SOM ( a mass-weighted age of material in the soil) with SOM *turnover time* (i.e., the flux-weighted age of material leaving the soils) can create large errors in the predicted dynamics of soil organic matter, for example if even a small fraction is more  $^{14}\text{C}$  depleted than the bulk material (i.e., giving the one-pool model a longer turnover time; Trumbore, 2009).

### 4.3 Microbial biomass

The isotopic composition of microbial biomass and microbial respired  $\text{CO}_2$  in the sandstone soil (0–5 cm) showed that nearly half the microbial biomass carbon was more than five years old and approximately 40 % of the carbon respired by microbes had been fixed more than five years ago. Bulk microbial biomass could have an older isotopic signature if microbes consume older SOC, for example recycling microbial biomass, or if a significant fraction of the microbial biomass is dormant or otherwise turns over very slowly. Two earlier studies based on the  $^{14}\text{C}$  value of microbial biomass or microbial respired  $\text{CO}_2$  (Kramer and Gleixner, 2006; Garnett et al., 2011) also found microbial biomass contained old C and interpreted that a component of the soil microbial community used older SOC as substrates. However, in our study the isotopic results suggest that use of older substrates was unlikely the sole explanation for older microbial C – in other words, it is unlikely that half the microbial biomass is supported by assimilating older SOC and half by new C. Specifically, 85 % of the microbial respiration came from only 10 % of the total soil C (the fast pool, with young isotopic signature). For the assimilation associated with the remaining 15 % of respired  $\text{CO}_2$  to support 50 % of the biomass, microbes assimilating old C would have to be at least three times as efficient in retaining assimilated C in biomass, or turn over at least three times more slowly.

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## 4.4 Implications

Recognizing that soil physical fractions are better described as comprising at least two functional pools of very different dynamics has three main mechanistic implications.

5 First, the light fraction does not contain all the easily decomposable organic matter in a soil sample, and is not itself all easily decomposable. Organic matter in the light fraction can be subject to different degrees of physical protection, because free and occluded (inter- and intra-aggregate particulate OM, respectively) light-fraction materials have been shown to incorporate  $^{14}\text{C}$  at different rates (Swanston et al., 2005). There is empirical evidence that there are chemically different kinds of organic carbon within the  
10 light fraction (Golchin et al., 1994). In addition to labile plant materials, occluded light fraction in forest, grassland, and arable soil contained more charred materials than the free light fraction (Brodowski et al., 2006). We offer as a hypothesis for a future study that for many soils there is a useful generalization that the light fraction comprises plant inputs in a spectrum of decay.

15 Second, and perhaps even more important, the dense fraction contains both strongly bound (and old) and weakly associated, fast cycling (and younger) organic matter. Similar conclusions were reached by sequential density fractionation combined with radiocarbon, NEXAFS, and thermal calorimetry (Kleber et al., 2011). This also confirms findings from coniferous forest soils, where acid hydrolysis separated the DF  
20 ( $\delta^{14}\text{C}$  value of  $-1\text{‰}$ ) into a decadal-cycling hydrolyzed fraction (74 % of DF C;  $\delta^{14}\text{C} = +25\text{‰}$ ) and slower-cycling non-hydrolyzable residue (26 % of DF C;  $\delta^{14}\text{C} = -74\text{‰}$ ; Trumbore and Zheng, 1996). Recent conceptual models for mineral-organic interactions are consistent with this observation. For example, Kleber et al. (2007) conceptualized SOM as having a heterogeneous range of amphiphilic properties, and capable  
25 of self-organization in a polar aqueous solution (water), suggesting that SOM sorbs to mineral surfaces with different exchange kinetics, including a component or outer layer that is only weakly stabilized.

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Third, depth had little influence on turnover of the fast-cycling pool, but strongly affected turnover in the slow-cycling pool of both fractions. Increasing soil depth corresponds to differences in decomposition environment such as pore space, aggregation, and oxygen availability, such that the sum of protective mechanisms is higher with depth (Rumpel and Kögel-Knabner, 2011). We infer that the turnover time of the slow cycling pool responds more to the protective environment than does the fast pool, likely because the mechanisms of stabilization are linked to the physical and chemical properties of the soil (von Lützow et al., 2006; Schmidt et al., 2011). In contrast, the finding that the turnover time of the fast pool was 3–10 yr regardless of depth or density fraction is consistent with having organic matter chemistry as the main constraint on decomposition rates of that pool. However, the finding is equally consistent with the existence of hot spots of decomposition, which can occur, for example, because of heterogeneity in soil structure (Nunan et al., 2003; Chabbi et al., 2009), rhizosphere influence (Cheng et al., 2003; Kuzyakov, 2010), and microbial predation (Griffiths, 1994).

Significant and efficient sequestration of C in soil will require ways to transfer C into the slow pool (which is stabilized by protective mechanisms). An increase in productivity or decrease in respiration from the fast pool alone will not be as effective, because, for example, a 50 % reduction in the decay constant for heterotrophic respiration would only double the stock of the small sized fast pool, and leave the large slow pool reservoir mostly unaffected.

Although the ideal SOM model might have a continuum of turnover times, there is a qualitative improvement in capturing SOM dynamics by moving from a one-pool model to a two-pool model for any component or physical fraction (three or more pools would be even better, but the biggest jump in predictive ability is between one and two pools). For process-level studies and carbon budgeting, it is worth the effort to look for situations that will allow constraining such a model. The most obvious opportunity is provided by elevated CO<sub>2</sub> experiments using OTC (open top chamber) or FACE (free air CO<sub>2</sub> enrichment) facilities. There have been 37 FACE experiments globally and more than 10 experiments are still running

([http://public.ornl.gov/face/global\\_face.shtml](http://public.ornl.gov/face/global_face.shtml)). Soils with the dual isotopic label from these experiments could be exploited to learn much more about the rates and controls of soil C cycling (e.g., Hopkins et al., 2012). In fact, it may be difficult to accurately capture the mechanisms of soil carbon stabilization or predict rates of carbon cycling without the ability to combine isotopic tracers with physical fractionations.

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**Table 1.** Isotopic signature of ambient/tank air and plant inputs used in the model.

Variable	Model Parameter	$\delta^{13}\text{C}$ (‰)	$\Delta^{14}\text{C}$ (‰)	Source	Remarks
Ambient Air ( $\text{CO}_2$ in ambient atmosphere)	Ambient Air	-8	Decrease from 136 (1991) to 100 (1996)	Hua and Barbeti (2004) (ambient air $^{14}\text{C}$ )	Year-specific $\delta^{14}\text{C}$ values used in the model.
Tank Air ( $\text{CO}_2$ added to elevated $\text{CO}_2$ chambers)	Tank Air	-35	-1000	Jackson et al. (1994) (tank air $^{13}\text{C}$ )	
Chamber Air ( $\text{CO}_2$ within elevated $\text{CO}_2$ chambers)	Chamber Air	-20	Decrease from -369 (1991) to -389 (1996)	Jackson et al. (1994) (chamber air $^{13}\text{C}$ )	For $^{14}\text{C}$ , mass balance using $f$ (0.444) and $\delta^{14}\text{C}$ of ambient air and tank air
Proportion of tank air in elevated $\text{CO}_2$ chambers	$f = 0.444$			Calculation by a two-member mixing model	Mass balance using $\delta^{13}\text{C}$ of Ambient Air, Tank Air and Chamber Air.
Air–plant discrimination factor against the heavier isotope	Ambient = $\Delta_A$	-20.6	-41.2	Jackson et al. (1994) ( $^{13}\text{C}$ discrimination factor)	Assume the $^{14}\text{C}$ discrimination is two times the $^{13}\text{C}$ discrimination.
	Elevated = $\Delta_E$	-21.6	-43.2		
$^{13}\text{C}$ and $^{14}\text{C}$ in plants	Ambient = $P_C$	-28.6	Same as the $\delta^{14}\text{C}$ value of the air inside the chambers		For $^{13}\text{C}$ , $P = \Delta + \text{air}$
	Elevated = $P_E$	-41.6			For $^{14}\text{C}$ , $P = \text{air}$

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**Table 2.** Mean carbon and nitrogen contents, C : N ratio, isotopic values, and carbon stocks of two density fractions. Values in parentheses are standard error,  $n = 10$  except  $\delta^{14}\text{C}$  is single value for composite of 10 samples.

	Light fraction						Dense fraction					
	C %	N %	C/N	$\delta^{13}\text{C}$ ‰	$\delta^{14}\text{C}$ ‰	C-stock $\text{g m}^{-2}$	C %	N %	C/N	$\delta^{13}\text{C}$ ‰	$\delta^{14}\text{C}$ ‰	C-stock $\text{g m}^{-2}$
<b>Sandstone 0–15 cm</b>												
Ambient	15.2(0.59)	0.90(0.03)	16.8	-28.6(0.1)	71.7	331(36)	0.86(0.04)	0.093(0.003)	9.3	-27.5(0.1)	10.9	1223(59)
Elevated	15.1(0.86)	0.87(0.02)	17.2	-32.7(0.4)	-91.5	354(35)	0.92(0.03)	0.099(0.003)	9.4	-28.5(0.1)	-35.6	1156(73)
<b>Sandstone 15–30 cm</b>												
Ambient	17.9(2.14)	0.93(0.05)	19.0	-28.2(0.2)	-16.7	126(14)	0.64(0.03)	0.074(0.003)	8.5	-26.7(0.1)	-65.4	922(30)
Elevated	16.1(1.04)	0.88(0.05)	18.3	-31.0(0.6)	-130	132(14)	0.63(0.02)	0.072(0.002)	8.6	-30.0(0.2)	-111	941(44)
<b>Serpentine 0–15 cm</b>												
Ambient	22.7(0.47)	1.42(0.04)	16.0	-28.8(0.1)	61.0	395(46)	1.46(0.07)	0.156(0.005)	9.3	-27.3(0.1)	-5.3	989(40)
Elevated	20.7(1.23)	1.33(0.08)	15.5	-33.7(0.3)	-80.0	370(29)	1.36(0.06)	0.152(0.007)	8.9	-28.4(0.1)	-32.8	989(42)

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**Table 3.** Turnover time ( $\tau$ , year) modeling results for the one pool model, which assumes that the soil physical fraction C has one homogeneous turnover time. Column header shows the data set used to constrain turnover time. There are two solutions for the modeling of the observed  $^{14}\text{C}$  value in elevated  $\text{CO}_2$  plots, hence two columns.

	Ambient $\text{CO}_2$ $^{14}\text{C}$	Elevated $\text{CO}_2$ $^{14}\text{C}$ , low value	Elevated $\text{CO}_2$ $^{14}\text{C}$ , high value	Elevated $\text{CO}_2$ $^{13}\text{C}$
<b>Light Fraction</b>				
Sandstone 0–15 cm	124	11	917	23
Sandstone 15–30 cm	406	9	1301	36
Serpentine 0–15 cm	141	11	812	18
<b>Dense Fraction</b>				
Sandstone 0–15 cm	275	13	455	114
Sandstone 15–30 cm	742	10	1102	420
Serpentine 0–15 cm	346	13	436	105

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**Table 4.** Turnover times ( $\tau$ , year) and its relative size (% of total carbon) for light (LF) and dense (DF) density fractions as modeled with data from the ambient  $\text{CO}_2$  treatment, the elevated  $\text{CO}_2$  treatment, or both. The data and constraints are for (A) ambient  $\text{CO}_2$  treatment, one constraint,  $^{14}\text{C}_{\text{ambient}}$ ; (B) elevated  $\text{CO}_2$  treatment, two constraints,  $^{14}\text{C}_{\text{elevated}}$  and  $^{13}\text{C}_{\text{elevated}}$ ; and (C) both ambient and elevated  $\text{CO}_2$  treatments modeled simultaneously with same turnover times, three constraints,  $^{14}\text{C}_{\text{ambient}}$ ,  $^{14}\text{C}_{\text{elevated}}$ , and  $^{13}\text{C}_{\text{elevated}}$ .  $C_{\text{new}}$  is based on  $^{13}\text{C}$  signatures of plant inputs and soils and calculated by Eq. (11). Sa, sandstone; Sp, serpentine.

		$C_{\text{new}}$	$C_{\text{fast}}$	$C_{\text{slow}}$		Flux-weighted	
		Size (%)	$\tau$ (year)	Size (%)	$\tau$ (year)	$\tau_{\text{effective}}$ (year)	
<b>A: Ambient <math>\text{CO}_2</math></b>							
LF	Sa 0–15	30	3.2	30	150	70	10.2
	Sa 15–30	21	3.2	23	654	77	13.7
	Sp 0–15	36	11.1	54	867	46	20.3
DF	Sa 0–15	7	2.8	7	304	93	35.6
	Sa 15–30	2	3.0	9	885	91	32.2
	Sp 0–15	7	16.2	9	460	91	133
<b>B: Elevated <math>\text{CO}_2</math></b>							
LF	Sa 0–15	30	2.7	28	153	72	9.2
	Sa 15–30	21	1.8	20	699	80	8.9
	Sp 0–15	36	11.3	56	865	44	20.0
DF	Sa 0–15	7	4.0	5	351	95	65.8
	Sa 15–30	2	1.6	1	1071	99	139
	Sp 0–15	7	15.0	14	466	86	89.5
<b>C: Both ambient and elevated <math>\text{CO}_2</math></b>							
LF	Sa 0–15	30	2.7	30	150	70	8.6
	Sa 15–30	21	3.2	23	654	77	13.7
	Sp 0–15	36	11.0	54	867	46	20.2
DF	Sa 0–15	7	3.0	7	304	93	37.9
	Sa 15–30	2	3.3	9	881	91	35.3
	Sp 0–15	7	15.6	9	460	91	129

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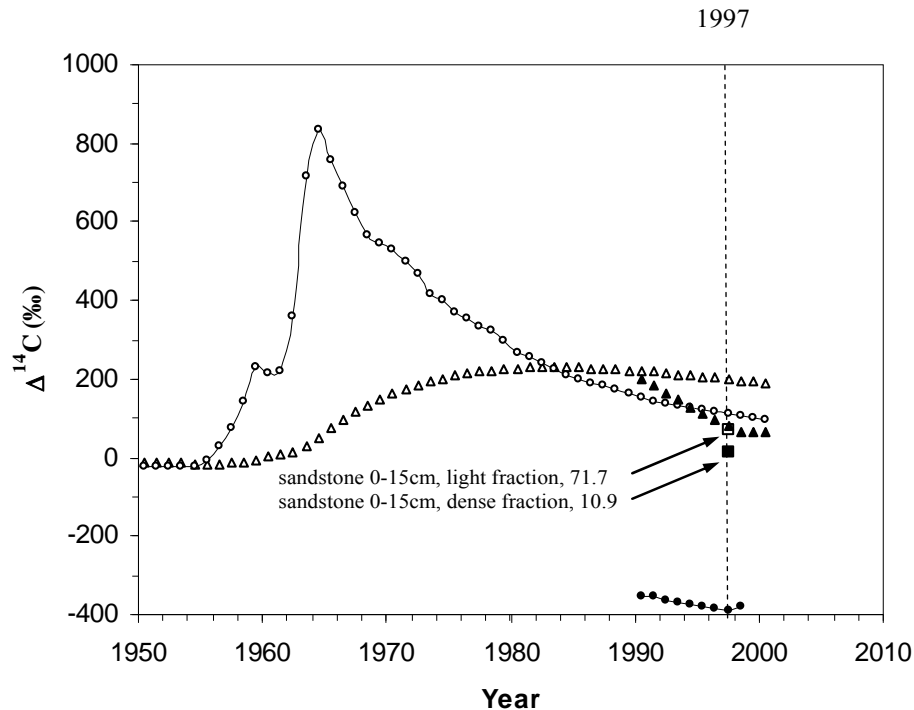
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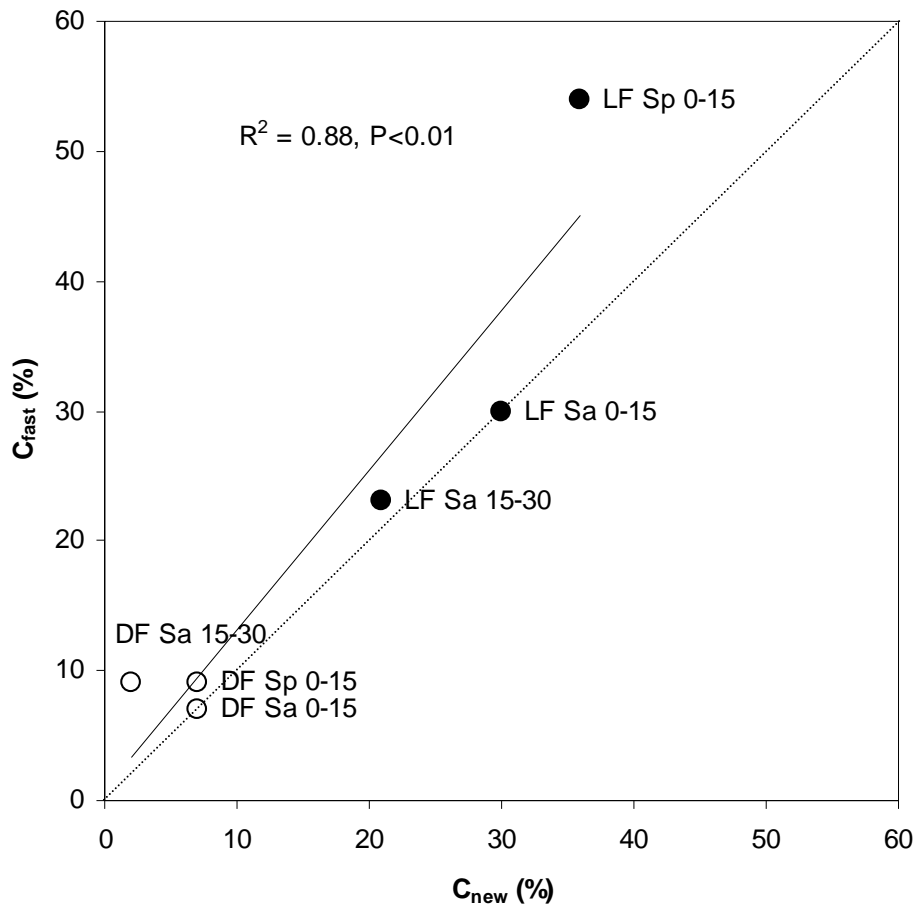


**Table 5.** Isotopic signatures of microbial biomass and biomass respiration measured for the sandstone topsoil (0–5 cm) sampled in April 1996.

Variable	Model parameter	$\delta^{13}\text{C}$ (‰)	$\delta^{14}\text{C}$ (‰)	Fraction modern	Source	Remarks
CO <sub>2</sub> respired during 10 day incubation from fumigated treatment	Ambient = $F_A$	-28	135	1.135	measured	Direct measurement
	Elevated = $F_E$	-35	-183	0.817		
CO <sub>2</sub> respired during 10 day incubation from unfumigated treatment	Ambient = $R_A$	-29	125	1.125	measured	Direct measurement
	Elevated = $R_E$	-37	-213	0.787		
Microbial Biomass	Ambient = $M_A$	-27	140	1.140	Eqs. (12–14)	Mass balance using CO <sub>2</sub> respired from fumigated and unfumigated treatments
	Elevated = $M_E$	-34	-162	0.838		



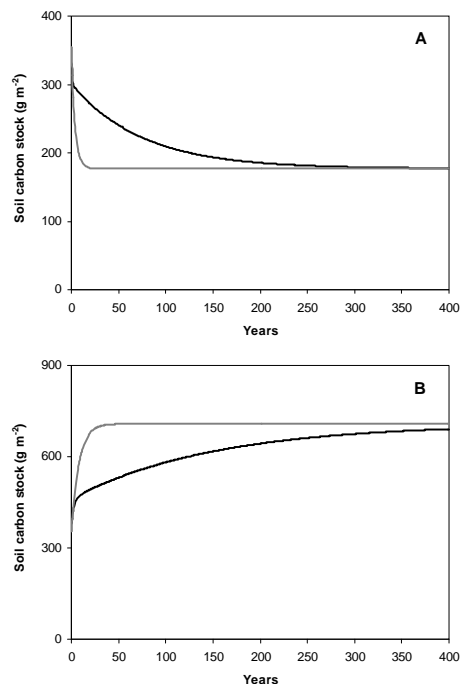
**Fig. 1.** Measured values of  $^{14}\text{CO}_2$  in the ambient air of the Northern Hemisphere (open circles) and  $^{14}\text{CO}_2$  in the air supplied to the elevated  $\text{CO}_2$  treatment (filled circles) compared to calculated  $^{14}\text{C}$  values of soil organic matter in the control (open triangles) and the elevated  $\text{CO}_2$  (filled triangles) treatments for an arbitrary turnover time of 30 yr. Arrow tips indicate measured  $\delta^{14}\text{C}$  C values in sandstone topsoil (0–15 cm) light (open square) and dense (filled square) fractions in the year of 1997.



**Fig. 2.** The % of each soil fraction that is new ( $C$  incorporated since the experiment began;  $C_{new}$ ) vs. the % of each soil fraction that is fast cycling ( $C_{fast}$ ).  $C_{new}$  explains 88% of the variation in  $C_{fast}$  ( $n = 6$ ,  $P < 0.01$ ). LF, light fraction; DF, dense fraction; Sa, sandstone; Sp, serpentine.

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**Fig. 3.** Change in soil C stock ( $\text{kg m}^{-2}$ ) after **(A)** doubling of decay rates (or 50 % decrease in turnover times), and **(B)** doubling of NPP inputs for the two-pool (black line) and one-pool (grey line) models. For the two-pool model, the turnover time data are for sandstone 0–15 cm Light Fraction, in which 30 % of the fraction has  $\tau = 2.7$  yr and 70 % has  $\tau = 150$  yr (Table 4). The one-pool model has a single turnover time (8.6 yr) and gives the same total soil  $\text{CO}_2$  flux ( $41.0 \text{ g m}^{-2} \text{ yr}^{-1}$ ) from a stock of  $354 \text{ g m}^{-2}$  in steady state as does the two-pool model.

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