

1 The turnover of carbon pools contributing to soil CO<sub>2</sub> and soil respiration in a  
2 temperate forest exposed to elevated CO<sub>2</sub> concentration

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1 **Abstract**

2 Soil carbon is returned to the atmosphere through the process of soil respiration,  
3 which represents one of the largest fluxes in the terrestrial C cycle. The effects  
4 of climate change on the components of soil respiration can affect the sink or  
5 source capacity of ecosystems for atmospheric carbon, but no current techniques  
6 can unambiguously separate soil respiration into its components. Long-term  
7 Free Air CO<sub>2</sub> Enrichment (FACE) experiments provide a unique opportunity to  
8 study soil C dynamics because the CO<sub>2</sub> used for fumigation has a distinct  
9 isotopic signature and serves as a continuous label at the ecosystem level. We  
10 used the <sup>13</sup>C tracer at the Duke Forest FACE site to follow the disappearance of  
11 C fixed before fumigation began in 1996 (pre-treatment C) from soil CO<sub>2</sub> and soil-  
12 respired CO<sub>2</sub>, as an index of belowground C dynamics during the first 8 years of  
13 the experiment. The decay of pre-treatment C as detected in the isotopic  
14 composition of soil-respired CO<sub>2</sub> and soil CO<sub>2</sub> at 15-cm, 30-cm, 70-cm and 200-  
15 cm soil depth was best described by a model having 1-3 exponential pools within  
16 the soil system. The majority of soil-respired CO<sub>2</sub> (71%) originated in soil C  
17 pools with a turnover time of about 35 days. About 55%, 50%, and 68% of soil  
18 CO<sub>2</sub> at 15, 30, and 70 cm respectively originated in soil pools with turnover times  
19 of less than 1 year. The rest of soil CO<sub>2</sub> and soil-respired CO<sub>2</sub> originated in soil  
20 pools that turn over at a decadal time scale. Our results suggest that a large  
21 fraction of the C returned to the atmosphere through soil respiration results from  
22 dynamic soil C pools that cannot be easily detected in traditionally defined soil  
23 organic matter standing stocks. Fast oxidation of labile C substrates may

- 1 prevent increases in soil C accumulation in forests exposed to elevated [CO<sub>2</sub>]
- 2 and may consequently result in shorter ecosystem C residence times.

## 1 **Introduction**

2           Current increases in atmospheric [CO<sub>2</sub>] have the potential to lead to  
3 imbalances between C uptake and C loss by terrestrial ecosystems, with  
4 consequent feedbacks to atmospheric CO<sub>2</sub> accumulation and global climate  
5 change. The amount of carbon in soils represents about two thirds of total  
6 ecosystem carbon (Schimel et al., 1994; Schlesinger, 1997) and soil organic  
7 matter (SOM) includes C pools with the slowest turnover rates in the terrestrial  
8 biosphere (Trumbore, 1997). Because of the magnitude of this C pool, soils  
9 have the potential to impact atmospheric [CO<sub>2</sub>] and, consequently, global  
10 climate. The heterogeneous nature of SOM complicates analyses of soil C  
11 turnover and the role of soils in determining the C source or sink capacity of  
12 terrestrial ecosystems with changing environmental conditions remains unclear.

13           Soil C is returned to the atmosphere through the process of soil respiration  
14 (R<sub>S</sub>), which represents one of the largest fluxes in the terrestrial C cycle  
15 (Schlesinger, 1977; Raich & Schlesinger, 1992; Raich et al., 2002). R<sub>S</sub> consists  
16 of autotrophic root respiration, as well as heterotrophic respiration associated  
17 with the decomposition of root-derived C, root and leaf litter, and SOM. SOM is a  
18 complex mixture of organic compounds with turnover times that range from days  
19 to millennia (Paul and van Veen, 1978), and SOM decomposition occurs  
20 heterogeneously as the sum of several components (Balesdent *et al.*, 1987;  
21 Melillo *et al.*, 1989). While radiocarbon measurements of SOM indicate that the  
22 average age of bulk soil is in the range of several hundred to several thousand  
23 years (O'Brien & Stout, 1978; Anderson & Paul, 1984), the majority of soil C

1 returned to the atmosphere through  $R_S$  has a mean residence time (MRT)  
2 ranging from years to decades (Harrison et al., 1993; Townsend et al., 1995;  
3 Trumbore et al., 1995; Gaudinski *et al.*, 2000; Trumbore, 2000). In order to  
4 account for some of the heterogeneity in SOM decomposition, most models  
5 designed to simulate SOM dynamics include several conceptual pools, which  
6 vary in their individual decomposition rates and the factors that control these  
7 rates (reviewed by Smith et al., 1997). The conceptual C pools described in  
8 models, however, cannot always be directly compared to measurable soil C  
9 compartments, making it difficult to validate experimentally how C transfer  
10 between pools affects SOM cycling (Six et al., 2002). Additionally, the C pools  
11 depicted in models may not be homogeneous in their turnover rates and  
12 assigning one decay constant to each compartment may not always be  
13 appropriate for determining rates of SOM cycling (Magid *et al.* 2002).

14 In soils, decaying organic compounds are transformed and stabilized  
15 according to their biochemical decomposability, as well as according to the  
16 degree of chemical and physical protection from decomposers provided by soils  
17 (Christensen, 1996). The decay rate of SOM that is physically or chemically  
18 protected may not be a function of its inherent decomposability, as soil aggregate  
19 formation may increase the MRT of the labile organic matter contained within  
20 aggregates (Oades, 1984; Elliott and Coleman, 1988; Jastrow et al., 1996; Kaiser  
21 & Guggenberger, 2003). Various chemical (e.g. Gleixner *et al.*, 1999, 2002) and  
22 physical (Christensen, 2001) soil fractionation techniques have been applied to  
23 isolating SOM pools according to their stability and turnover rates. Soil

1 fractionation analyses combined with stable (Balesdent *et al.*, 1988; Six *et al.*,  
2 2001; Van Groenigen *et al.*, 2002; Lichter *et al.*, 2005) and radioactive C isotope  
3 tracers (Gaudinski *et al.*, 2000; Trumbore, 2000) have also provided useful tools  
4 for determining the ages and decomposition rates of soil C compartments. While  
5 the turnover rates of individual SOM fractions are often more informative in  
6 studies of soil C dynamics than turnover rates of bulk SOM, they may not always  
7 compare well with the age of soil CO<sub>2</sub> fluxes. The age of soil CO<sub>2</sub> usually reflects  
8 that of a labile soil C pool that turns over relatively quickly and may not be easily  
9 detected in SOM stocks. Analyses of the C isotope composition of soil CO<sub>2</sub> and  
10 soil-respired CO<sub>2</sub> can therefore be useful for identifying and quantifying the active  
11 soil C pool, which represents the majority of soil C returned to the atmosphere,  
12 bypassing the limitations introduced by chemical and physical protection of SOM.

13         The development of the Free Air CO<sub>2</sub> Enrichment (FACE) technology  
14 (Hendrey *et al.* 1999) has allowed studies of the C cycle of intact ecosystems  
15 exposed to elevated [CO<sub>2</sub>]. The fumigation CO<sub>2</sub> at FACE sites can also function  
16 as a continuous ecosystem <sup>13</sup>C label, providing a means for studying SOM  
17 dynamics. Studies from FACE sites report incorporation of labeled C into SOM  
18 pools but no significant soil C accumulation (Leavitt *et al.* 2001; Schlesinger &  
19 Lichter 2001; Hoosbeek *et al.* 2004; van Groenigen *et al.* 2003; Lichter *et al.*  
20 2005; but see Matamala *et al.* 2003). In this study, we used the ecosystem <sup>13</sup>C  
21 tracer at the Duke Forest FACE site (Chapel Hill, NC) to determine relative  
22 contributions and turnover times of the soil C pools contributing to soil CO<sub>2</sub> and  
23 R<sub>s</sub>. We used the rate of disappearance of C assimilated before CO<sub>2</sub> fumigation

- 1 began in 1996 from soil CO<sub>2</sub>, as an index of belowground C dynamics during the
- 2 first 8 years of the FACE experiment.

## 1 **Methods**

### 2 *Site Description*

3           The Free Air CO<sub>2</sub> Enrichment (FACE) experiment at the FACTS-1  
4 Research Site (Chapel Hill, NC) is composed of six 30-m diameter plots, situated  
5 in an intact *Pinus taeda* plantation (Hendrey et al., 1999). The three treatment  
6 plots are fumigated with CO<sub>2</sub> to maintain an atmospheric [CO<sub>2</sub>] that is  
7 approximately 200 μL L<sup>-1</sup> above ambient, or about 567 ± 4 μL L<sup>-1</sup> (averaged from  
8 1996-2004; K. Lewin and R. Nettles, pers. comm.); the three control plots are  
9 fumigated with ambient air only. Continuous fumigation of these plots began on  
10 August 27 1996, when the trees were 15 years old. CO<sub>2</sub> fumigation is switched  
11 off when temperatures are below 5°C and when sustained wind speed exceeds 5  
12 ms<sup>-1</sup>. Fumigation is turned off due to weather conditions for about 12% of the  
13 year (averaged from 1996-2004), mostly during the winter when photosynthetic  
14 rates are low (K. Lewin and R. Nettles, pers. comm.). Starting December 16  
15 2002, fumigation was switched to daytime only.

16           The site was cleared of a mixed forest in 1981, burned in 1982, and  
17 established as a loblolly pine plantation in 1983, with 3-year-old seedlings  
18 planted at 2.4 x 2.4 m spacing. Since that time, a number of hardwood species  
19 have become established in the understory, the most abundant of which are *Acer*  
20 *rubrum*, *Cercis canadensis*, *Liquidambar styraciflua*, *Liriodendron tulipifera* and  
21 *Ulmus alata*. Soils at the site are a low fertility Ultic Alfisol of the Enon Series,  
22 derived from igneous rock with an acidic, well-developed profile with mixed clay  
23 mineralogy. Soil bulk density is 1.07 g cm<sup>-3</sup> at 0-20 cm depth and fine roots are

1 mostly found in the upper 20 cm of the soil profile. Mean annual temperature is  
2 15.5°C and mean annual precipitation is 1140 mm.

3

#### 4 *Ecosystem <sup>13</sup>C tracer*

5 The CO<sub>2</sub> used for fumigation at FACTS-1 is depleted in <sup>13</sup>C ( $\delta^{13}\text{C} \approx -$   
6  $43.1 \pm 0.6 \text{ ‰ SE}$ ) vs. PDB, where  $\delta^{13}\text{C} = [(R_{\text{sample}} - R_{\text{reference}})/R_{\text{reference}}] \times 1000$  and  
7  $R = ^{13}\text{C}/^{12}\text{C}$ . When CO<sub>2</sub> concentration is elevated by 200  $\mu\text{L L}^{-1}$  in the treatment  
8 plots, the  $\delta^{13}\text{C}$  of atmospheric CO<sub>2</sub> decreases from about  $-8$  to  $-20 \pm 0.4 \text{ ‰}$ . As  
9 a consequence, new needles and fine roots produced under FACE have a  $\delta^{13}\text{C}$   
10 of  $-41.8 \pm 0.3 \text{ ‰}$  and  $-39.2 \pm 0.8 \text{ ‰}$  compared to  $\delta^{13}\text{C}$  of  $-29.9 \pm 0.2 \text{ ‰}$  and  $-$   
11  $27.6 \pm 0.2 \text{ ‰}$  at ambient conditions, respectively (Matamala et al., 2003; L.  
12 Taneva, unpublished data, 2003). Therefore, the fumigated forest plots have  
13 been exposed to a continuous ecosystem <sup>13</sup>C label since the beginning of the  
14 CO<sub>2</sub> treatment in 1996. From the incorporation of this isotopic label into soil and  
15 soil-respired CO<sub>2</sub>, we were able to follow the decomposition of pre-treatment soil  
16 C in the treatment plots during the first 8 years of CO<sub>2</sub> fumigation.

17

#### 18 *Soil CO<sub>2</sub> Concentration*

19 The CO<sub>2</sub> concentration in the soil pore space was measured on samples  
20 drawn from four gas wells each at 15-, 30-, and 70-cm depths per plot and two  
21 wells at 200-cm depth per plot as described in Andrews and Schlesinger (2001).  
22 Each gas well was placed in a 10-cm diameter hole so that the PVC-pipe  
23 bottoms rested at the target depth and holes around the pipe were backfilled with

1 original soil in reverse order of removal. The gas wells were open at the bottom  
2 and closed at the top with a two-holed rubber stopper, connected directly to the  
3 soil surface with two 0.6-cm diameter Kynar<sup>®</sup> plastic tubes. Samples were drawn  
4 directly from gas wells through a magnesium perchlorate (Mg(ClO<sub>4</sub>)) water trap  
5 into a field-portable Infra Red Gas Analyzer (EGM-1, PP Systems, Inc., Haverhill,  
6 Massachusetts).

7

### 8 *Gas Sample Collection and Stable Carbon Isotope Analysis*

9 Gas samples were collected for stable carbon isotope analysis from the  
10 same soil gas wells as described above. Gas samples were collected for  
11 analysis from wells into evacuated (10<sup>-5</sup> Pa) 75-cm<sup>3</sup> stainless steel gas cylinders  
12 (Whitey<sup>®</sup>), sealed with Nupro<sup>®</sup> bellows valves equipped with Kel-F<sup>®</sup> stem tips.  
13 Samples of soil-respired CO<sub>2</sub> were collected from respiration chambers  
14 according to the methods described in Andrews & Schlesinger (2001). Soil and  
15 soil-respired CO<sub>2</sub> were purified by cryogenic extraction (as in Trueman and  
16 Gonzalez-Meler, 2005) and analyzed for their isotopic composition by using  
17 either a SIRA II (VG Isotech, UK) or a Finnegan MAT Delta plus XL (Bremen,  
18 Germany) Isotope Ratio Mass Spectrometer, located at either Duke University or  
19 the University of Illinois at Chicago stable isotope facilities.

20

### 21 *Mixing model and end-member determination*

22 Soil-respired CO<sub>2</sub> (growing season only) and soil CO<sub>2</sub> at each depth was  
23 partitioned into C that was photosynthetically fixed since the beginning of CO<sub>2</sub>

1 fumigation (referred to as 'post-treatment' C) and C assimilated under ambient  
2 [CO<sub>2</sub>] before fumigation started (referred to as 'pre-treatment' C), according to  
3 the following two end-member mixing equation:

$$4 \quad \delta^{13}\text{C}_{\text{soil CO}_2} = f \cdot \delta^{13}\text{C}_{\text{pre-tr}} + (1-f) \cdot \delta^{13}\text{C}_{\text{post-tr}} \quad (1)$$

5 where  $\delta^{13}\text{C}_{\text{soil CO}_2}$  is the measured  $\delta^{13}\text{C}$  of soil CO<sub>2</sub> at each depth at time  $t$ ,  
6  $\delta^{13}\text{C}_{\text{post-tr}}$  is the end-member for post-treatment C,  $\delta^{13}\text{C}_{\text{pre-tr}}$  is the end-member for  
7 pre-treatment C at time  $t$  and  $f$  represents the fraction of pre-treatment C in soil  
8 CO<sub>2</sub> at time  $t$ .

9 The  $\delta^{13}\text{C}_{\text{pre-tr}}$  is determined by directly measuring  $\delta^{13}\text{C}_{\text{soil CO}_2}$  in the control  
10 plots of the experiment at time  $t$ . This measured value incorporates respiration  
11 from both recalcitrant and labile soil C pools under ambient CO<sub>2</sub> conditions.  
12 Because the  $\delta^{13}\text{C}$  of recalcitrant soil C pools has little or no seasonal variation  
13 (Balesdent & Mariotti, 1996), any seasonal variability in  $\delta^{13}\text{C}$  of soil CO<sub>2</sub> in the  
14 control plots will be due to differences in the signature of labile soil C pools (i.e.  
15 root respiration), reflecting seasonal fluctuations in photosynthetic discrimination  
16 (see Farquhar et al., 1989). Because the  $\delta^{13}\text{C}$  of the atmosphere in the enriched  
17 plots was changed by a constant value (E) at the beginning of the experiment  
18 and because photosynthetic discrimination against <sup>13</sup>C is the same under  
19 ambient and elevated [CO<sub>2</sub>] (due to lack of photosynthetic acclimation and  
20 conserved C<sub>i</sub>/C<sub>a</sub> between ambient and elevated [CO<sub>2</sub>] plots (Ellsworth, 1999)),  
21 the difference in  $\delta^{13}\text{C}$  of new photosynthate in the control and treatment plots  
22 also equals E. Therefore, the end-member for the  $\delta^{13}\text{C}$  of soil CO<sub>2</sub> in the

1 enriched plots ( $\delta^{13}\text{C}_{\text{post-tr}}$ ) can be derived by subtracting E from the measured  
2  $\delta^{13}\text{C}_{\text{pre-tr}}$  and equation (1) can be rearranged as follows:

$$3 \quad f = (\delta^{13}\text{C}_{\text{soil CO}_2} - \delta^{13}\text{C}_{\text{post-tr}})/E \quad (2)$$

4 where E was found to be 12‰. We follow the fraction of pre-treatment C ( $f$ ),  
5 instead of the absolute amount of pre-treatment C, remaining in soil CO<sub>2</sub> and R<sub>S</sub>  
6 over time because measurements of soil CO<sub>2</sub> concentration and soil CO<sub>2</sub> isotopic  
7 composition were not made on the same sample or at the same time. Soil CO<sub>2</sub>  
8 concentrations show great seasonal variation (Andrews & Schlesinger, 2001;  
9 Bernhardt *et al.*, in press) and therefore, we were not able to follow the absolute  
10 decrease of pre-treatment C in soil CO<sub>2</sub> throughout each year.

11 A potential error that can be associated with this model is the possibility  
12 that the addition of post-treatment C may decrease the value of  $\delta^{13}\text{C}_{\text{soil CO}_2}$   
13 without a change in the concentration of pre-treatment C in the soil, which would  
14 affect the value of  $f$ . In order to address this potential error,  $\delta^{13}\text{C}_{\text{soil CO}_2}$  was  
15 allowed to vary by 1‰ (affecting ~10% of  $f$ ). This sensitivity analysis also  
16 accounts for possible changes in photosynthetic discrimination, which would  
17 affect E, even though it is unlikely that such differences between the control and  
18 treatment plots occur.

19

## 20 *SOM dynamics and pool partitioning*

21 The disappearance of pre-treatment C from soil CO<sub>2</sub> follows an  
22 exponential decay function according to the equation  $f = e^{-kt}$ , where  $f$  represents  
23 the fraction of pre-treatment C remaining over time and  $k$  is the decay constant

1 for pre-treatment C. The MRT of pre-treatment C in soil CO<sub>2</sub> can be calculated  
2 from this equation as  $-1/k$ . The MRT calculated from this single-pool model  
3 gives an integrated estimate of the turnover times of all the C pools contributing  
4 to soil CO<sub>2</sub> and soil-respired CO<sub>2</sub>. Because soil CO<sub>2</sub> originates in several C  
5 pools that turn over at different rates, we applied a model used in radiochemistry  
6 for the analysis of mixtures of independently decaying radionuclides, to  
7 determine the relative contributions and turnover rates of individual soil C pools  
8 contributing to soil CO<sub>2</sub> and soil-respired CO<sub>2</sub>. This approach consists of splitting  
9 the original exponential decay curve into up to 3 components and successively  
10 subtracting longer-lived components from the original curve to get both the  
11 fractions and the half lives of all components of the mixture (Overman & Clark,  
12 1960; Friedlander *et al.*, 1981). A similar approach has also been used to  
13 determine isotope residence times in animals after changes in diet (see Ailyffe *et*  
14 *al.* 2004 and references therein).

15       When the disappearance of pre-treatment C from soil CO<sub>2</sub> was plotted  
16 over time, the number of different C pools contributing to soil CO<sub>2</sub> were  
17 determined by log-transforming the y-axis to emphasize the breaking points  
18 between pools and then using the least-square method (0.05) to identify which  
19 points belong to each curve. An exponential decay curve was fitted to the last  
20 portion of the original curve to determine the contribution and turnover rate of the  
21 slowest C pool. The contribution of this pool was then subtracted from the  
22 original curve and the remaining portion was analyzed in the same way to  
23 determine the contribution and turnover rate of the remaining C pools. The C

1 pools were referred to as 'slow', 'intermediate' and 'fast' based on their relative  
2 turnover times. This approach can be applied to mixtures of a small number of  
3 components ( $n < 4$ ) with half lives that differ by at least a factor of 2 (Friedlander *et*  
4 *al.*, 1981). Because of the limitations of this approach in the number of soil C  
5 pools contributing to soil CO<sub>2</sub> that we were able to identify, the turnover time  
6 reported for each pool represents an average turnover time of all the sub-pools  
7 comprising it. Another limitation to this approach is that it cannot be applied to  
8 soil CO<sub>2</sub> in the control plots at FACTS-1 because there is no <sup>13</sup>C tracer under  
9 ambient CO<sub>2</sub> conditions. The rate of disappearance of pre-treatment C from soil  
10 CO<sub>2</sub> and soil-respired CO<sub>2</sub> should be the same in the treatment and control plots,  
11 unless the decomposition of pre-treatment C in the treatment plots has been  
12 affected by tree growth under elevated [CO<sub>2</sub>]. If ecosystem exposure to elevated  
13 [CO<sub>2</sub>] has affected the decomposition of pre-treatment C, then the results of our  
14 analyses are an overestimate of soil C turnover times.

## 1 **Results**

### 2 *Soil CO<sub>2</sub> concentration*

3           The annual average CO<sub>2</sub> concentrations in the treatment plots at FACTS-1  
4 are consistently higher than in the control plots at all soil depths, however the  
5 difference is not statistically significant (Fig. 1; Bernhardt *et al.*, in press).

6 Despite higher CO<sub>2</sub> concentrations, the absolute amount of pre-treatment C in  
7 soil CO<sub>2</sub> is decreasing in the treatment plots since fumigation began (Fig. 1).

8 During the first 8 years of FACE, the amount of pre-treatment C in soil CO<sub>2</sub>  
9 declined from 100% to 39% at 15 cm, 30% at 30 cm, 42% at 70 cm and 23% at  
10 200 cm soil depth relative to control plots (Fig. 1). These results compare well  
11 with our analyses of the turnover times of soil C pools contributing to soil CO<sub>2</sub>,  
12 using the fraction of pre-treatment C remaining over time, as determined from  
13 equation (2) (presented below).

14

### 15 *Soil-respired CO<sub>2</sub>*

16           The  $\delta^{13}\text{C}$  of growing season (April to October) soil-respired CO<sub>2</sub> in the  
17 treatment plots at FACTS-1 declined sharply after the start of CO<sub>2</sub> fumigation,  
18 reflecting incorporation of recent photosynthate into soil CO<sub>2</sub> efflux. The  
19 integrated turnover time of pre-treatment C in soil-respired CO<sub>2</sub>, calculated using  
20 a single-pool exponential model, is  $246 \pm 65$  days (Table 1). The change in  
21 isotopic signature of growing season soil-respired CO<sub>2</sub>, however, can be used to  
22 identify two C pools contributing to R<sub>S</sub> (Fig. 2): a C pool with a fast turnover time  
23 of  $35 \pm 7$  days that contributed  $71.1 \pm 4.6$  % to soil-respired CO<sub>2</sub> and a C pool

1 with a relatively slow turnover time of  $11.5 \pm 2.9$  years that contributed  $29.0 \pm 3.3$   
2 % to soil-respired  $\text{CO}_2$  (Table 1).

3 Even though the absolute amount of pre-treatment C (Fig. 1) and the  
4 fraction of pre-treatment C calculated from equation (2) decline at comparable  
5 rates, it is possible that occasional increases in the release of post-treatment C  
6 can result in an overestimate of  $f$ . If the fraction of pre-treatment C remaining  
7 over time was consistently underestimated by 10% due to the addition of post-  
8 treatment C (see Methods section), maximizing the potential error in the analysis,  
9 the turnover times of the fast and slow pool in  $R_S$  would change from 35 to 36  
10 days and from 11.5 to 19.3 years, respectively.

11

12 *Soil  $\text{CO}_2$  at 15, 30, 70, and 200 cm depth.*

13 The  $\delta^{13}\text{C}$  of soil  $\text{CO}_2$  in the treatment plots at FACTS-1 declined  
14 exponentially with time at all sampling depths, reflecting incorporation of labeled  
15 C into soil C pools. When the decay of pre-treatment C from soil  $\text{CO}_2$  is treated  
16 as a single-pool exponential model, the average turnover time of pre-treatment C  
17 in soil  $\text{CO}_2$  is  $4.2 \pm 0.6$  years at 15 cm,  $2.3 \pm 0.3$  years at 30 cm,  $3.2 \pm 0.4$  years  
18 at 70 cm, and  $5.3 \pm 0.5$  years at 200 cm depth (Table 1).

19 The observed changes in the  $\delta^{13}\text{C}$  of soil  $\text{CO}_2$  at each sampling depth can  
20 be used to calculate the turnover times and relative contributions of the soil C  
21 pools contributing to soil  $\text{CO}_2$ . The decay of pre-treatment C as detected in soil  
22  $\text{CO}_2$  at 15, 30, 70 and 200 cm is best described by a model having 1-3  
23 exponential soil C pools (Fig. 3). At 15-cm depth, the change in isotopic

1 signature of soil CO<sub>2</sub> is consistent with a pool having a fast turnover time of 10 ±  
2 2 days that contributed 9.1 ± 0.7 % to soil CO<sub>2</sub> (Fig. 3A). A C pool with an  
3 intermediate turnover time of 268 ± 55 days contributed 46.2 ± 6.3 % to soil CO<sub>2</sub>  
4 at this depth and C pool with a relatively slow turnover time of 38.1 ± 41.2 years  
5 contributed the remaining 44.6 ± 6.8 % to soil CO<sub>2</sub> at 15 cm depth (Fig. 3A). The  
6 large range in the turnover time of the slow C pool may be due to the presence of  
7 several C pools within it that have different individual turnover rates. If not  
8 enough time has passed for all the C pools to have turned over at least once,  
9 then they will not be detected with this approach and will appear as one pool with  
10 a weighted average turnover time of all the C pools within it. In order to estimate  
11 the potential error in the calculation of *f* due to the addition of post-treatment C,  
12 the turnover times of the C pools at 15 cm were also calculated when the fraction  
13 of pre-treatment C remaining over time was consistently underestimated by 10%.  
14 When underestimating *f* in this way, the turnover times of the fast, intermediate  
15 and slow pools at this depth changed from 10.0 to 9.8 days, from 268 to 245  
16 days, and from 38 to 33 years, respectively.

17         The C isotope signature of soil CO<sub>2</sub> at 30 cm was consistent with two  
18 exponential soil C pools, with 50.5 ± 2.2 % of soil CO<sub>2</sub> originating in a C pool with  
19 an intermediate turnover time of 186.2 ± 21.7 days and 48.1 ± 2.5 % of soil CO<sub>2</sub>  
20 coming from a C pool with a relatively slow turnover time of 7.6 ± 0.8 years (Fig.  
21 3B). If the fraction of pre-treatment C remaining over time was consistently  
22 underestimated by 10% due to the addition of post-treatment C, the turnover time

1 of the intermediate pool changed from 186 to 200 days, whereas the turnover  
2 time of the slow C pool changed from 7.6 to 14.6 years.

3         The change in isotopic signature of soil CO<sub>2</sub> at 70 cm was similarly  
4 consistent with two soil C pools, each contributing  $67.7 \pm 2.4$  % and  $32.9 \pm 3.0$  %  
5 to CO<sub>2</sub> at this depth, with turnover times of  $355.6 \pm 30.0$  days and  $48.0 \pm 38.5$   
6 years, respectively (Fig. 3C). As was the case at 15 cm depth, the CO<sub>2</sub>  
7 originating in the slow pool at 70 cm may reflect a large range in turnover rates  
8 from several C pools within this slow pool. If the fraction of pre-treatment C  
9 remaining over time was consistently underestimated by 10% due to the addition  
10 of post-treatment C, the turnover times of intermediate and slow pools at 70 cm  
11 changed from 355 to 350 days and from 48 to 65 years, respectively.

12         This approach does not have the sensitivity to detect more than one soil C  
13 pools contributing to soil CO<sub>2</sub> at 200-cm depth, possibly because not enough  
14 time has passed for a significant amount of pre-treatment C to be replaced by the  
15 labeled C in the slow pools at this depth (Fig. 3D). The integrated turnover time  
16 for pre-treatment C in soil CO<sub>2</sub> at 200 cm from a single-pool exponential model is  
17  $5.3 \pm 0.5$  years, reflecting the range in turnover rates of all the C pools  
18 contributing to soil CO<sub>2</sub> at this depth. If the fraction of pre-treatment C remaining  
19 over time was consistently underestimated by 10% due to the addition of post-  
20 treatment C, the integrated turnover time of these pools would change from 5.3  
21 to 6.0 years.

## 1 Discussion

2           The majority of soil-respired CO<sub>2</sub> (71%) and soil CO<sub>2</sub> (50-68%) down to a  
3 depth of 70 cm in this temperate forest originated in soil C pools with a turnover  
4 time of less than 1 year (Table 1). Studies using <sup>13</sup>C and <sup>14</sup>C tracers show that  
5 soil C released to the atmosphere within a year of photosynthetic assimilation  
6 represents 59% of R<sub>S</sub> in a temperate deciduous forest (Gaudinski *et al.* 2000),  
7 50-60% in boreal forests and up to 80% in tropical forests (Trumbore 2000;  
8 Giardina *et al.* 2004). The measured turnover times for the forest floor, roots and  
9 several physical and chemical SOM fractions in this ecosystem range from about  
10 4 to 48 years (Matamala *et al.* 2003; Lichter *et al.* 2005) and do not match the  
11 fast turnover times of the soil C pools in soil CO<sub>2</sub> we detect in this study. Our  
12 results suggest that a large fraction of the C returned to the atmosphere through  
13 R<sub>S</sub> results from dynamic soil C pools that cannot be easily detected in  
14 traditionally defined SOM standing stocks. Fast oxidation of labile C substrates  
15 may prevent increases in soil C accumulation in forests exposed to elevated  
16 [CO<sub>2</sub>] and may consequently result in shorter ecosystem C residence times.

17           During the first eight years of ecosystem exposure to elevated [CO<sub>2</sub>], soil-  
18 respired CO<sub>2</sub> and soil CO<sub>2</sub> at all depths show incorporation of the <sup>13</sup>C tracer. If  
19 the disappearance of pre-treatment C from soil CO<sub>2</sub> is treated as the result of a  
20 single process, the integrated turnover time of pre-treatment C in soil CO<sub>2</sub> is  
21 calculated as less than 6 years at the soil surface and at soil depths of up to 2 m  
22 (Table 1), comparable with an average turnover time of soil C in R<sub>S</sub> of 4 years  
23 reported for other temperate forests (Gaudinski *et al.* 2000). The rate of

1 incorporation of the  $^{13}\text{C}$  tracer, however, suggests that several processes interact  
2 on different time scales to bring about the observed changes in the isotopic  
3 composition of soil  $\text{CO}_2$ . About 71% of soil-respired  $\text{CO}_2$  comes from soil C  
4 pools that turn over in approximately 35 days and 9% of soil  $\text{CO}_2$  at 15 cm comes  
5 from a pool with a turnover time of 10 days (Table 1). This C pool is likely to  
6 represent the turnover of recent photosynthate within the root and rhizosphere  
7 system. Rapid incorporation of recent photosynthate into  $R_S$  has been previously  
8 reported in this forest (Andrews *et al.* 1999). The isotopic composition of  
9 ecosystem respiration and  $R_S$  in other forests has similarly been reported to  
10 correspond to photosynthate assimilated during the previous 2-10 days (Ekblad  
11 & Högberg, 2001; Bowling *et al.*, 2002). Furthermore, substantial reductions in  
12  $R_S$  5-10 days after experimental tree girdling or coppicing have also been  
13 reported (Bhupinderpal-Singh *et al.* 2003; Trueman & Gonzalez-Meler, 2005),  
14 suggesting that photosynthetic C is respired by roots and root-associated  
15 heterotrophs within several days of fixation.

16 In addition to recent photosynthate, mycorrhizal and bacterial turnover  
17 may also explain a fraction of these fast soil C pools. Experimental girdling was  
18 shown to reduce microbial biomass by 32% 1-3 months after the supply of  
19 current photosynthate to the roots was terminated (Högberg & Högberg, 2002).  
20 Ostle *et al.* (2003) used  $^{13}\text{CO}_2$  pulse labeling to determine the residence time of  
21 current photosynthate in the soil microbial biomass from the turnover of labeled  
22 RNA, which they calculated as 15-20 days. The complete turnover time of  
23 microbial biomass in the soil has been estimated to be about 1 year (Paul &

1 Juma, 1981; Paul, 1984); however fractions within the microbial biomass C pool  
2 may turn over much more rapidly (Lundquist *et al.* 1999). Microbial turnover may  
3 therefore explain a fraction of both the fast and intermediate C pools that we  
4 detect in this study.

5 A soil C pool with a turnover time of 6 months to 1 year contributes about  
6 46%, 51% and 68% to soil CO<sub>2</sub> at 15, 30, and 70 cm, respectively (Table 1).  
7 Since most fine roots in this forest are found in the upper 20 cm of the soil profile  
8 (Matamala & Schlesinger, 2000), root and rhizosphere activity is unlikely to  
9 explain the origin of a C pool with a relatively fast turnover rate at 30- and 70-cm  
10 soil depths. The nature of this C pool can possibly be explained by downward  
11 transfer of labile dissolved organic carbon (DOC). In forest ecosystems, the flux  
12 of DOC from the forest floor to the mineral soil has been estimated to be about  
13 115-500 kg C ha<sup>-1</sup> yr<sup>-1</sup> (Guggenberger & Zech, 1993; Curie *et al.*, 1996; Michalzik  
14 & Matzner, 1999; Solinger *et al.*, 2001; Kaiser *et al.*, 2001) and therefore, part of  
15 this intermediate C pool is possibly comprised of leachates from the O horizon.  
16 In a similar *Pinus taeda* forest in South Carolina, Richter *et al.* (1999) reported  
17 that approximately 33% of soil C inputs in the top 15 cm of mineral soil are from  
18 downward movement of DOC. The turnover time of the forest floor in the  
19 treatment plots at FACTS-1 is estimated to be 4.2 years (Lichter *et al.* 2005),  
20 which is comparable with estimates of 2-5 years reported for temperate  
21 deciduous forests (Gaudinski *et al.* 2000); however, the forest floor is composed  
22 of C pools that decompose and are leached to the mineral soil at different rates  
23 and may turn over much more rapidly (reviewed by Kalbitz *et al.* 2000). Qualls &

1 Haines (1992) reported that 14-33% of the DOC leached from the organic  
2 horizon in a deciduous forest was metabolized over 134 days, with most  
3 oxidation occurring in the first 20 days. Similarly, Boyer & Groffman (1995)  
4 report that 10-25% of water-extractable soil C was metabolized over 14 days and  
5 Qualls & Bridgham (2005) report that 32% of DOC leached from the litter layer in  
6 their study had a turnover time of 7 days, with the remaining 68% turning over in  
7 1.6 years. Therefore, it is likely that DOC decomposition may also partially  
8 contribute to the C pool with a fast turnover time of 35 days, which represents  
9 71% of  $R_S$ . If a two end-member mixing model is applied to this labile pool  
10 detected in  $R_S$ , with the end-members being the turnover times of 10 and 268  
11 days for the fast and intermediate C pools detected in soil  $CO_2$  at 15 cm (Table  
12 1), then the intermediate C pool may represent up to 20% of the active C pool  
13 detected in  $R_S$ . The remaining 51% of this active pool is likely composed of root  
14 and rhizosphere respiration, which compares well with previous studies reporting  
15 that root respiration contributes about 55% to  $R_S$  (Andrews *et al.*, 1999; Hui &  
16 Luo, 2004).

17 Our results show that about 29% of soil-respired  $CO_2$  during the growing  
18 season originates in soil C pools that turn over in about 12 years (Table 1). Our  
19 results are consistent with those for other forests, where 41% of  $R_S$  in a  
20 deciduous forest was reported to come from soil C with a mean age of several  
21 years (Gaudinski *et al.* 2000) and about 40-50% of  $R_S$  in boreal and temperate  
22 forests was shown to originate from soil C more than a year old (Trumbore,  
23 2000). In this study, 45%, 48% and 33% of soil  $CO_2$  at 15, 30, and 70 cm soil

1 depths respectively comes from soil C pools that turn over in the range of several  
2 years to several decades (Table 1). In a boreal forest, about 9-22% of  
3 heterotrophic respiration was shown to be derived from decomposition of SOM  
4 more than several decades old (Trumbore & Harden, 1997). Recalcitrant soil C  
5 that is very old may dominate the average age of bulk SOM throughout the soil  
6 profile (O'Brien & Stout, 1978), but its contribution to soil CO<sub>2</sub> and R<sub>s</sub> is usually  
7 very small (Paul *et al.* 2001), which may prevent detection of these recalcitrant C  
8 pools in soil CO<sub>2</sub>. The slow C pool that we detect with our analysis is likely to be  
9 very heterogeneous and its turnover rate possibly represents an average of the  
10 turnover rates of all the C pools within it. The forest at FACTS-1 has been  
11 exposed to the <sup>13</sup>C label for only 8 years and this time period may not have been  
12 sufficient for the label to become incorporated into longer-lived soil C pools,  
13 which would impede our ability to detect them with this approach. In order to  
14 separate two C pools with different turnover times with the approach presented  
15 here, one of the pools must have turned over at least once. If not enough time  
16 has passed for that to occur, then two discrete pools will appear as one pool with  
17 a turnover time that is an average value of the turnover times of the pools within  
18 it. Our analysis is therefore more sensitive to identifying C pools with relatively  
19 fast turnover times.

20 Our results suggest that a substantial proportion of soil C is returned to the  
21 atmosphere within a year of photosynthetic assimilation (Table 1). The  
22 remainder of soil CO<sub>2</sub> and soil-respired CO<sub>2</sub> originates in C pools with turnover  
23 times of several years to several decades. Changes in the turnover rate of fast

1 soil C pools under elevated [CO<sub>2</sub>] may not substantially affect atmospheric [CO<sub>2</sub>];  
2 however, changes in the pool size of these soil components may alter the cycling  
3 of more recalcitrant soil C by priming their decomposition (Zak et al., 1993; 2000;  
4 Fontaine et al., 2003; Trueman & Gonzalez-Meler, 2005). It should be noted that  
5 the soil C turnover times reported here are for a forest growing under elevated  
6 [CO<sub>2</sub>] and may be equal to or faster than the turnover time for soil C under  
7 current ambient CO<sub>2</sub> conditions.

8         The isotopic composition of soil and ecosystem respiration has been used  
9 to infer the contribution of terrestrial ecosystems to the isotopic composition of  
10 atmospheric CO<sub>2</sub>, as a means to characterize net CO<sub>2</sub> uptake by oceans and the  
11 terrestrial biosphere (Tans et al., 1993; Ciais et al., 1995; Fung et al., 1997,  
12 Randerson et al., 2002). The isotopic composition of ecosystem respiration is a  
13 function of the relative contributions of all of its components and thus, of the  
14 average turnover times of carbon in plant biomass and soils, since plant and  
15 heterotrophic respiration utilize C substrates assimilated at different times. Using  
16 the mechanistic terrestrial model Sim-CYCLE (Ito and Oikawa, 2002) and the  
17 observed variability in atmospheric δ<sup>13</sup>C and [CO<sub>2</sub>], Ito (2003) inferred an  
18 average ecosystem C residence time of 15-25 years in North American  
19 temperate forests, most of the uncertainty residing in soil carbon. Similarly, Malhi  
20 et al. (1999) and Robinson (2004) estimated the average turnover time of forest  
21 floor and soil C in temperate forests to be 10-15 years. In our study, the average  
22 turnover time of the C pools contributing to R<sub>S</sub> and soil CO<sub>2</sub> ranged from 0.7 to  
23 5.3 years (Table 1). Our results indicate that the average turnover time of active

- 1 soil C may be lower than the estimates used or derived from models, which may
- 2 lead to underestimation of soil C decomposition rates and overestimation of net
- 3 C uptake in temperate forests.

1 **Conclusions**

2 In this study, we determined the turnover times and relative contributions of  
3 different soil C pools to soil respiration and soil CO<sub>2</sub> in a temperate forest, by  
4 using a continuous 8-year long atmospheric CO<sub>2</sub> label. Our results indicate that  
5 a substantial proportion of soil C at the Duke Forest FACE site is returned to the  
6 atmosphere within a year of photosynthetic assimilation. Our approach was  
7 more useful in identifying these faster C pools, which dominate soil respiration,  
8 than traditional soil C pool separation techniques, which are more sensitive to the  
9 slower and more recalcitrant C pools. Such fast turnover rates of soil C, as  
10 detected in soil CO<sub>2</sub> and soil respiration, do not agree well with estimates of  
11 forest soil C turnover used in terrestrial C models, suggesting possible  
12 underestimation of soil C decomposition rates and overestimation of net  
13 ecosystem productivity in temperate forests by models.

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1 Table 1.  
2 The soil C pools contributing to soil-respired CO<sub>2</sub> and soil CO<sub>2</sub> at 15, 30, 70, and  
3 200 cm soil depth. The contributions and turnover times of the different soil C  
4 pools are reported as derived from a single-pool model, as well as from the  
5 multiple-pool approach illustrated in Figures 2 and 3. The terms ‘fast’,  
6 ‘intermediate’ and ‘slow’ refer to the relative turnover time of these pools at each  
7 depth. The r<sup>2</sup> values are for the exponential curves fitted to each section of the  
8 original exponential decay curve, from which the turnover and relative  
9 contribution of each pool was determined. The number of points used to identify  
10 each pool ranged from 4-27.  
11

Depth	Contribution ± SE (%)	Turnover time ± SE	R <sup>2</sup>
<b>Soil-respired CO<sub>2</sub></b>			
Single pool model	100.0 ± 13.3	245.8 ± 65.1 d	0.31
Fast pool	71.0 ± 4.6	35.2 ± 7.5 d	0.98
Slow Pool	29.0 ± 3.3	11.5 ± 2.9 yr	0.49
<b>15 cm</b>			
Single pool model	100.0 ± 5.5	4.2 ± 0.6 yr	0.15
Fast pool	9.1 ± 0.7	10.0 ± 1.8 d	0.98
Intermed. Pool	46.2 ± 6.3	268.2 ± 54.5 d	0.75
Slow pool	44.6 ± 6.8	38.1 ± 41.2 yr	0.03
<b>30 cm</b>			
Single pool model	100.0 ± 5.8	2.3 ± 0.3 yr	0.67
Intermed. Pool	50.5 ± 2.2	186.2 ± 21.7 d	0.95
Slow pool	48.1 ± 2.5	7.6 ± 0.8 yr	0.78
<b>70 cm</b>			
Single pool model	100.0 ± 5.3	3.2 ± 0.4 yr	0.64
Intermed. Pool	67.7 ± 2.4	355.6 ± 30.0 d	0.95
Slow pool	32.9 ± 3.0	48.0 ± 38.5 yr	0.07

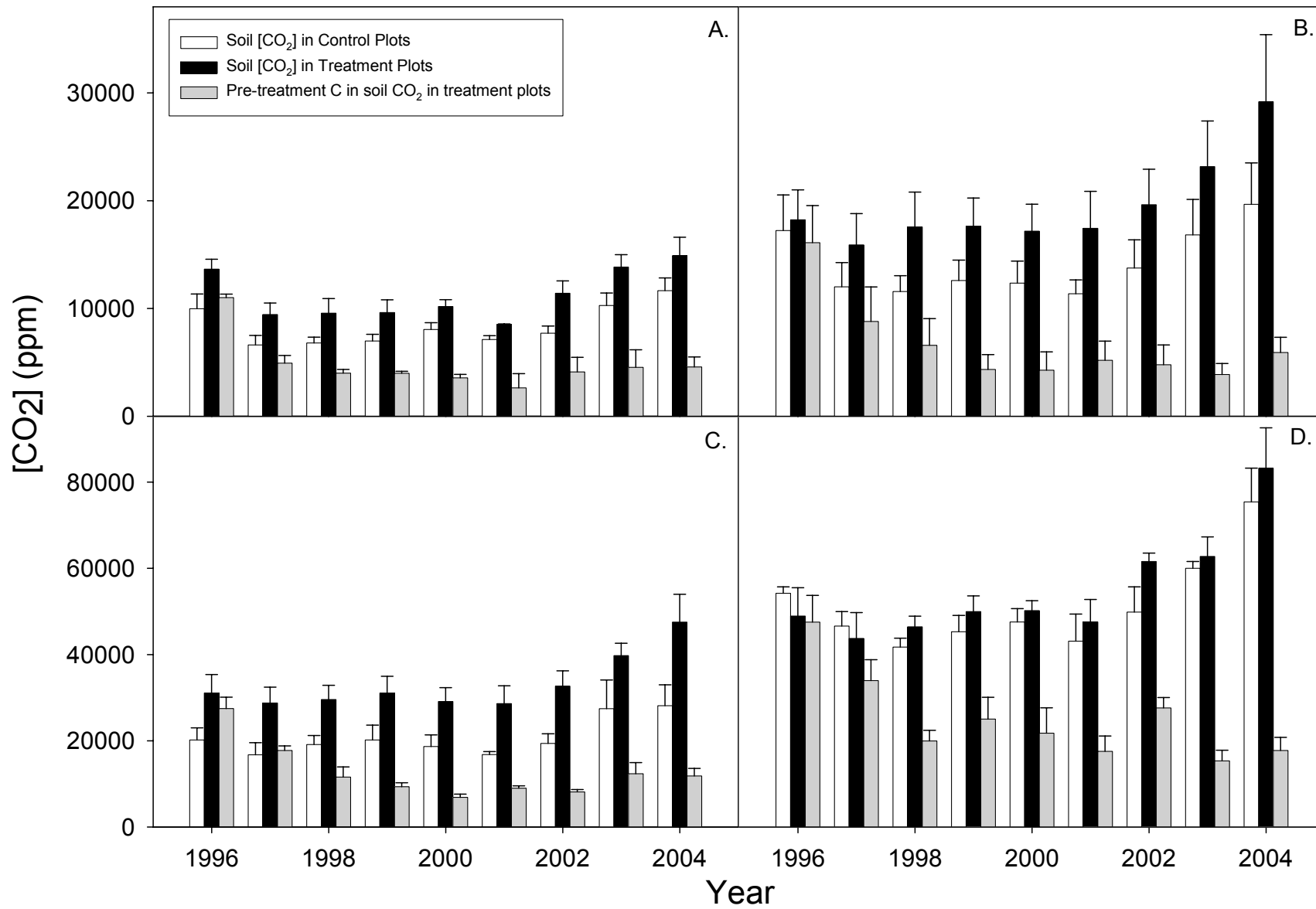
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**200 cm**

Single pool model	$100.0 \pm 4.8$	$5.3 \pm 0.5$ yr	0.84
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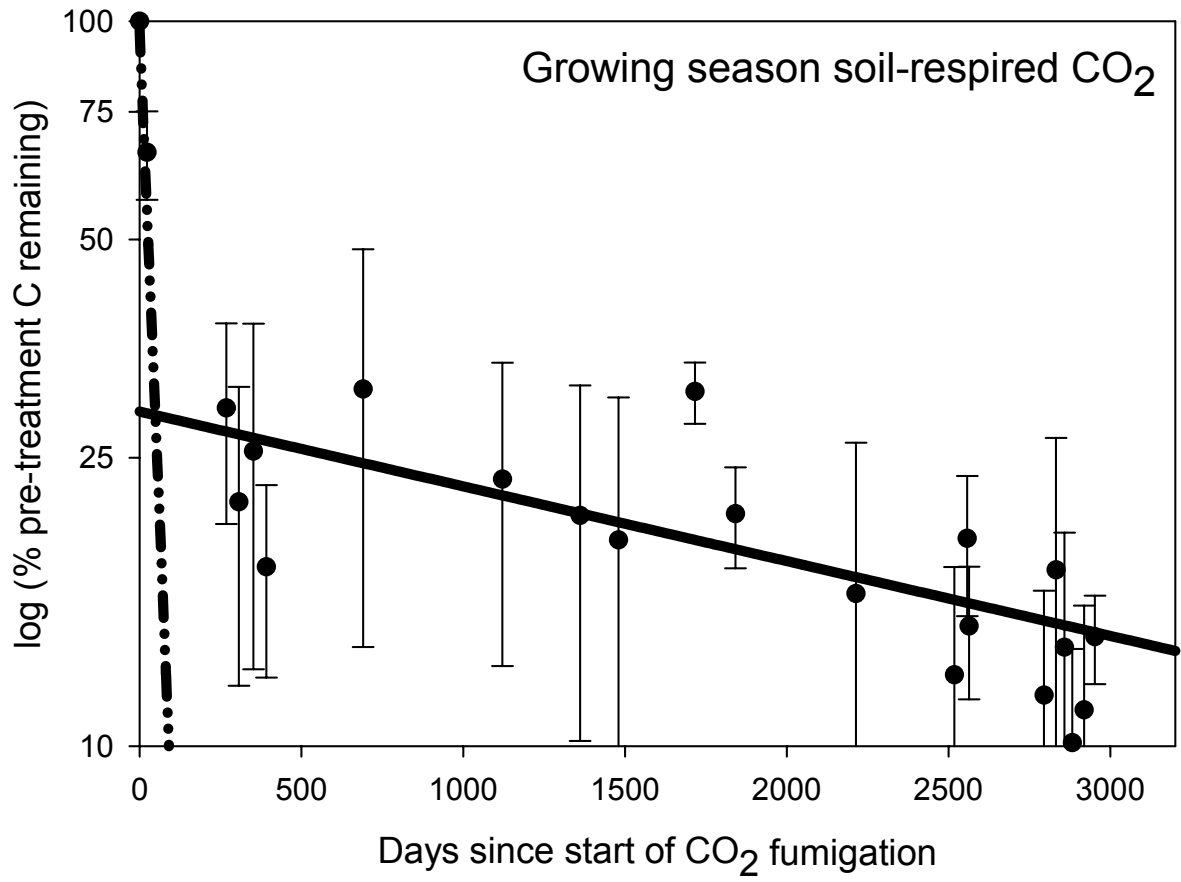
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1 Figure 1.

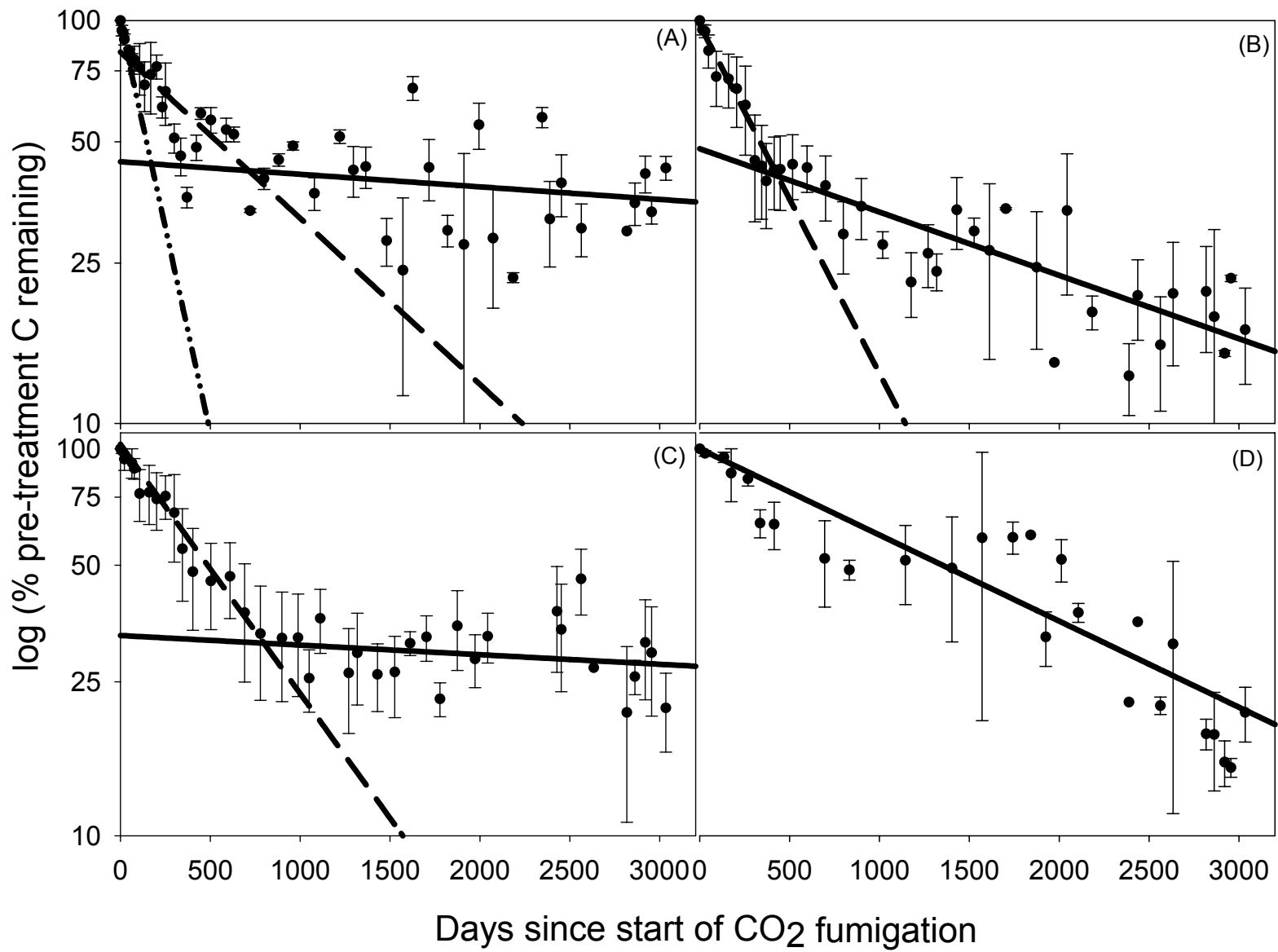


1 Figure 2.

2



1 Figure 3.



1 Figure 1.  
2 Annual average values of the CO<sub>2</sub> concentration in control and treatment plots,  
3 as well as the amount of pre-treatment C in soil CO<sub>2</sub> in the treatment plots at (A)  
4 15 cm, (B) 30 cm, (C) 70 cm, and (D) 200 cm depth. Bars show SE around the  
5 mean (n=3). The amount of pre-treatment C in soil CO<sub>2</sub> in the treatment plots  
6 was determined from the annual average [CO<sub>2</sub>] and annual average δ<sup>13</sup>C of soil  
7 CO<sub>2</sub> at each depth. Note difference in scale between the upper and lower  
8 panels.

9

10 Figure 2.

11 The proportion of pre-treatment C remaining in growing season soil-respired CO<sub>2</sub>  
12 since the beginning of CO<sub>2</sub> fumigation. Data points represent the average value  
13 ± standard error of treatment plots (n=3). The solid line represents the C pool  
14 with the relatively slow turnover time and the dashed line represents the C pool  
15 with the relatively fast turnover time.

16

17 Figure 3.

18 The proportion of pre-treatment C remaining in soil CO<sub>2</sub> at (A) 15 cm, (B) 30 cm,  
19 (C) 70 cm, and (D) 200 cm depth. Data points represent the average value ±  
20 standard error of treatment plots (n=3). The solid line represents the C pool with  
21 the slowest turnover time. The dashed line represents the C with an intermediate  
22 turnover time and the dashed/dotted line represents the C pool with the fastest  
23 turnover time detected at each depth.