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Initial characterization of processes of soil carbon stabilization using forest stand-level radiocarbon enrichment

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Abstract

Although the rates and mechanisms of soil organic matter (SOM) stabilization are difficult to observe directly, radiocarbon has proven an effective tracer of soil C dynamics, particularly when coupled with practical fractionation schemes. To explore the rates of C cycling in temperate forest soils, we took advantage of a unique opportunity in the form of an inadvertent stand-level ^{14}C-labeling originating from a local industrial release. A simple density fractionation scheme separated SOM into inter-aggregate particulate organic matter (free light fraction, free LF), particulate organic matter occluded within aggregates (occluded LF), and organic matter that is complexed with minerals to form a dense fraction (dense fraction, DF). Minimal agitation and density separation was used to isolate the free LF. The remaining dense sediment was subjected to physical disruption and sonication followed by density separation to separate it into occluded LF and DF. The occluded LF had higher C concentrations and C:N ratios than the free LF, and the C concentration in both light fractions was ten times that of the DF. As a result, the light fractions together accounted for less than 4% of the soil by weight, but contained 40% of the soil C in the 0–15 cm soil increment. Likewise, the light fractions were less than 1% weight of the 15–30 cm increment, but contained more than 35% of the soil C. The degree of SOM protection in the fractions, as indicated by $\Delta^{14}C$, was different. In all cases the free LF had the shortest mean residence times. A significant depth by fraction interaction for ^{14}C indicates that the relative importance of aggregation versus organo-mineral interactions for overall C stabilization changes with depth. The rapid incorporation of ^{14}C label into the otherwise depleted DF shows that this organo-mineral fraction comprises highly stable material as well as more recent inputs.

Keywords: Soil organic carbon; Stabilization; Forest soil; Density fractionation; Soil organic matter turnover

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1. Introduction

Carbon stabilization in forest soil organic matter (SOM) occurs concurrently with destabilization (Sollins et al., 1996), and large changes in the resident C in SOM may appear as only small net changes in bulk C values. Soil organic matter is protected by a variety of mechanisms, both physical and chemical, which results in SOM pools with different residence times (Oades, 1988; Torn et al., 1997). Although it is difficult to directly observe the processes of stabilization, carbon isotopes can give insight into rates of C cycling. Radiocarbon has proven an effective tracer of soil C at annual to decadal timescales because of the global spike in atmospheric $^{14}CO_2$ caused by thermonuclear weapons testing during the early 1960s (Goh et al., 1977; Trumbore, 1993; Trumbore and Zheng, 1996). However, the sensitivity of the bomb spike as a tracer is lower each year as the annual rate of atmospheric change declines (Levin and Hesshaimer, 2000). In addition, its application to some questions is limited because the same enrichment level applies to all ecosystems, making it hard to find an experimental control.

Recently, an unplanned ^{14}C release created a pulse label that can be used to study a range of questions in C cycling in a temperate forest region of the United States. In the summer of 1999, a large pulse of $^{14}CO_2$ was released near Oak Ridge, Tennessee, presumably from a local incinerator (Trumbore et al., 2002). The photosynthetic uptake of the $^{14}CO_2$ created a pulse label of ^{14}C in plant biomass of the local forest. There was a gradient in enrichment, with sites near the source experiencing about three times the level of the bomb spike (enriched sites), and those further away receiving much less (near-background sites). This stand-level isotopic label presents unique opportunities for studying different processes in belowground carbon cycling in some detail.

Fractionating soils allows isotopic and elemental analysis to be applied to specific portions of organic matter, increasing both the sensitivity to detect change and the ability to ascribe results to a particular process or category of the organic matter (Trumbore, 1993; Hungate et al., 1996; Trumbore and Zheng, 1996). A challenge in SOM fractionation however is to develop procedures that produce fractions that represent functionally different soil C pools while being simple enough to link to ecosystem models. In the basic procedure, the material that floats to the surface of a dense liquid is called the light fraction, and the material that sinks to the bottom is called the heavy or dense fraction (Greenland and Ford, 1964; Sollins et al., 1999). The density of the liquid is initially adjusted to maximize the recovery of particulate organic matter with high C concentration in the light fraction. In comparison to the dense fraction, the light fraction is characterized as chemically and visually more plant- or litter-like (Spycher et al., 1983; Golchin et al., 1994a; Amalfitano et al., 1995; Gregorich et al., 1996), and has been shown to be more sensitive to management (Dalal and Mayer, 1986; Compton and Boone, 2000). Organic C in the dense fraction (DF) is more closely associated with minerals and typically more stable in situ (Trumbore and Zheng, 1996; Gaudinski et al., 2000). Golchin et al. (1994a) adapted the method to better represent the complexities of soil structure by separating a "free" light fraction (free LF) from between aggregates and an "occluded" light fraction (occluded LF) from within aggregates. Carbon-13 NMR analyses of the free LF and occluded LF indicated that the occluded LF was somewhat more humified and presumably older. Baisden et al. (2002b) used ^{14}C to estimate the residence times of the free LF and three occluded light fractions (Golchin et al., 1994b) from grassland soils. They concluded that there was no appreciable difference among the three mineral-associated fractions, but found that the free light fraction had a shorter residence time than the occluded or mineral-associated carbon.

Our objectives were to (1) use the gradient of ^{14}C enrichment in the lands surrounding Oak Ridge National Laboratory, known as the Oak Ridge Reservation (ORR) to assess the effectiveness of the three-pool density fractionation procedure; and (2) to investigate C cycling and stabilization in those soils. Sites were selected with two levels of labeling: near-background sites and enriched sites. We considered the soils at the near-background site to be indicative of the system prior to the 1999 ^{14}C pulse. We used the results from the enriched site to gauge the progression of the recent ^{14}C pulse through soil organic matter. We judged a successful method to be one that would adequately discriminate meaningful C pools in the absence of a pulse label, as well as show sensitivity to

changes in those pools as elevated levels of ^{14}C moved through the system.

2. Materials and methods

2.1. Site characteristics

The Enriched Background Isotope Study (EBIS) was established in the autumn of 2000 on the Oak Ridge Reservation (ORR), in the U.S. Department of Energy's National Environmental Research Park near Oak Ridge, Tennessee (Trumbore et al., 2002). The mean annual precipitation at ORR is 1358 mm and mean annual temperature is 14 °C (Johnson and Van Hook, 1989). All EBIS research plots are located on upper slope, ridge positions in the upland oak forest type (*Quercus* spp.; *Acer* spp.; *Carya* spp.) with scattered pine (*Pinus echinata* and *Pinus virginiana*) and mesophytic hardwoods (*Liriodendron tulipifera*, *Fagus grandifolia*). The ages of the over-story trees cover a broad range from about 40–150 years, and the maximum canopy height is approximately 26 m. Maximum leaf area index is typically about 6 m^2 m^{-2}.

The whole-system background ^{14}C-enrichment, described previously by Trumbore et al. (2002), was not experimentally applied, but rather originated from an industrial source, such as hazardous waste incinerators, in the vicinity of ORR. There appear to have been numerous small releases beginning in 1995, punctuated by a large release between June and August of 1999 (Fig. 1). According to the record of ^{14}C in tree-ring cellulose, the releases prior to 1999 affected only the west end of the ORR (TVA site). The large 1999 release raised atmospheric levels at both sites but had a larger effect on the west end than the east (Walker Branch site). Labeled $^{14}CO_2$ was fixed by vegetation and incorporated into roots, leaves, and non-structural storage pools (Gaudinski, 2001; Trumbore et al., 2002). Measurements in the summer of 2000 revealed a gradient of ^{14}C-enrichment, highest in the western ORR and dropping to near-background in the eastern ORR.

Permanent plots were established during Winter 2000 in the western and eastern ORR. Eight 7×7 m plots were located at each of four sites. The sites were located on Inceptisols derived from shale or Ultisols derived from dolomitic parent materials. In the present work we only consider the Ultisols. These are deep, highly weathered soils with a 10–15-cm thick A horizon above a well-developed EB horizon and multiple Bt subsoil horizons. The clay fraction is dominated by kaolinite with lesser quantities of hydroxy-Al interlayered vermiculite.

In the present study, we analyze samples from 0–15 and 15–30-cm soils at an eastern (Walker Branch) and western site (TVA) of the ORR. Eight samples were taken in March 2001 from each depth increment at

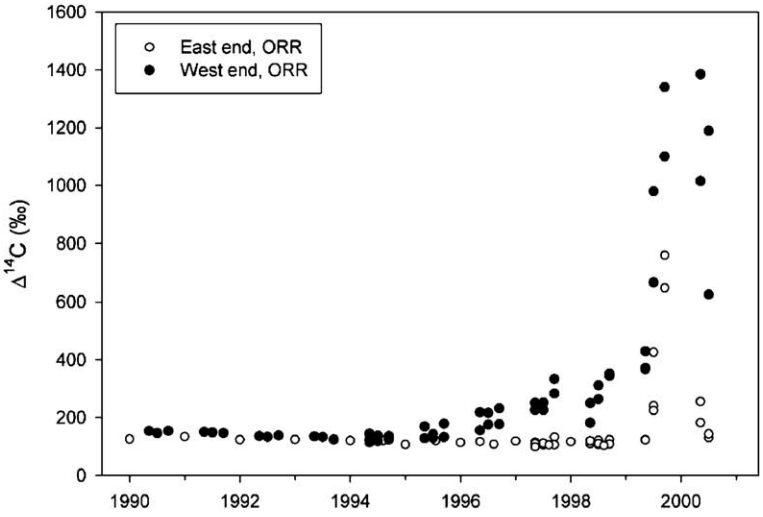

Fig. 1. $\Delta^{14}C$ from tree ring cellulose from in or near the eastern end of the Oak Ridge Reservation (open symbols) and the western end of the Reservation (closed symbols). Adapted from Trumbore et al. (2002).

each site, for a total of 32 bulk soil samples. Soil was collected with a 28-cm and a 10-cm diameter core sampler, respectively, and stored at −20 °C until further processing. Upon thawing, soils were sieved to <2 mm, sorted by hand to remove roots, and finally oven-dried at 105 °C.

Subsequent to collection of these samples, a litter manipulation study was initiated by applying highly ^{14}C-enriched (971±12‰) or near-background (222±1‰) litter, both collected in summer 2000, to the plots in a random design. In future studies, the litter treatments can be used to investigate the influence of litter versus root input of carbon (and ^{14}C) into soil organic carbon pools.

2.2. Density separation

Three density fractions were separated from bulk soil following the method of Golchin et al. (1994a): free light fraction, occluded light fraction, and dense fraction. About 100 mL of sodium polytungstate (NaPT, $Na_6[H_2W_{12}O_{40}]$, Sometu-US, Van Nuys, California) of 1.70 g mL^{-1} was added to 20 g oven-dried soil in a centrifuge bottle, and the bottle was inverted gently until the soil was wetted. After sitting for 45 min, the mixture was centrifuged in a bucket rotor for 45 min at 3600 rpm. Floating materials (free LF) were aspirated from the centrifuge bottle, then rinsed with double de-ionized H_2O (di H_2O) on a 0.8 μm polycarbonate filter (Whatman Nuclepore Track Etch Membrane). Sediment and NaPT remaining in the centrifuge bottle were mixed for 1 min using a benchtop mixer (G3U05R, Lightnin, New York, NY) at 1700 rpm. The blade was rinsed over the bottle, and the mixture sonicated in an ice bath for 3 min at 70% pulse for a total input of 200 J mL^{-1} (Branson 450 Sonifier, Danbury, CT). Debris were rinsed with NaPT from the sonicator probe into the bottle, and the mixture was centrifuged as before for 45 min. Floating materials (occluded LF) were very fine, and prone to clouding the supernatant with even small perturbations. If the supernatant was clouded, the bottle was allowed to sit up to 12 h before the occluded LF was aspirated. The separated occluded LF was also rinsed with di H_2O on a 0.8 μm polycarbonate filter. The sediment remaining in the centrifuge bottle (dense fraction, DF) was rinsed by repeatedly aspirating the supernatant, adding di H_2O, shaking, and centrifuging at 7500 rpm for 25 min. Fractions were dried for 24 h at 105 °C. Sample splits of the bulk soil were re-dried at 105 °C to correct for moisture accumulation during storage. We present all values on an oven-dry basis. Weights were measured and tracked to the nearest 0.0001 g.

2.3. Elemental analysis

Samples were analyzed for total C, total N, and fraction modern ^{14}C. The total C and N values for the density fractions were obtained from a Carlo Erba 2100 C/N elemental analyzer. Total C and N values for the bulk soil were obtained from a LECO CN-2000 elemental analyzer. Both were calibrated against standards traceable to the National Institute of Standards and Technology (Gaithersburg, MD). Radiocarbon values were measured on the Van de Graaff FN accelerator mass spectrometer (AMS) at the Center for Accelerator Mass Spectrometry, Lawrence Livermore National Laboratory. In preparation for AMS analysis, samples were combusted in evacuated, sealed tubes in the presence of CuO and Ag, then reduced onto iron powder in the presence of H_2 (Vogel et al., 1984). Splits of combusted sample were taken for ^{13}C analysis from each type of fraction from both sites and depths for correction of the AMS values, and reporting in Δ^{14}C notation (Stuiver and Polach, 1977).

2.4. Data analysis

The Walker Branch and TVA sites were considered separately, each as a split plot design. Soil increment was the main plot, and density fraction the subplot. Analyses of variance (ANOVA's) were carried out in SAS (SAS Institute Inc., Version 8.2) using the Mixed procedure. Residuals were examined for normality and constant variance, and if necessary transformed using natural logarithm or square-root transformations. Specifically, for Walker Branch, ^{14}C, C/N ratios, C and N concentrations, and soil C distribution in the density fractions were transformed with natural logarithm prior to accepting the ANOVA results. Likewise, for TVA, C:N ratios and C concentrations were transformed with square root, and N concentrations were transformed with natural logarithm. If ANOVA results were significant, pre-planned com-

Table 1
Mass recovery, C and N concentrations, and distribution of soil C in density fractions in the 0–15 cm and 15–30 cm soil increments of Walker Branch

	Mass recovery (% of bulk soil)	C[a] (g kg^{-1})	N[a] (g kg^{-1})	C/N	Distribution of C (% of bulk soil)[b]
0–15 cm					
Bulk soil	NA	24.89±2.20	0.97±0.04	25.5±1.5	NA
Free LF	2.17±0.37	299.79±7.94	8.85±0.20	34.1±1.7	25.52±2.44
Occl LF	1.08±0.11	388.57±9.31	9.31±0.40	42.3±2.0	16.74±0.80
DF	96.74±0.47	12.46±0.54	0.72±0.03	17.3±0.6	49.69±2.47
Total recovery	99.95±0.20	NA	NA	NA	91.83±0.82
15–30 cm					
Bulk soil	NA	5.89±0.51	0.17±0.02	35.6±2.6	NA
Free LF	0.44±0.05	326.31±8.57	6.91±0.42	45.5±2.1	23.84±1.86
Occl LF	0.24±0.02	360.93±2.38	6.53±0.63	57.2±3.7	14.93±1.19
DF	99.31±0.08	2.89±0.25	0.24±0.02	12.4±0.8	51.93±4.93
Total recovery	99.91±0.54	NA	NA	NA	85.92±2.47

Values are ±1 SE.
NA=not applicable.
[a] g kg^{-1} of fraction.
[b] Percentage of total soil C found in a given fraction.

parisons were made using least-squares differences within the Mixed model. All references to statistical differences between treatments are based upon a significance level of 0.05, although *p*-values between 0.05 and 0.1 are presented and discussed.

3. Results

3.1. Walker Branch (Near-background site)

Specific mass recoveries, C and N concentrations, and soil C distribution are located in Table 1. Carbon was at least 10 times more concentrated in the light fractions than in the dense fraction. As a result, the light fractions contained a disproportionate amount of the total soil C. In the upper soil, the free LF was only 2% of the soil mass, but contained 25% of the soil C. Likewise, the occluded LF was only 1% of the soil mass, but contained over 16% of the soil C. In contrast, the DF made up nearly 97% of the soil by mass, but contained only 50% of the soil C. The distribution of soil C in the fractions did not change significantly with depth (Table 2), although the relative mass of the light fractions four times greater in the upper soil. The remaining soil C was not

Table 2
Analysis of variance (ANOVA) results for main effects and interactions of soil variables at Walker Branch arranged and tested according to split-plot designs

Variable	Depth×Fraction			Depth			Fraction		
	df[a]	*F*	P	*df*[a]	*F*	P	*df*[a]	*F*	P
$\Delta^{14}C$	2,28	6.5	0.0005	1,14	33	NA	2,28	57	NA
C	2,25	185	<0.0001	1,14	157	NA	2,25	4368	NA
N	2,26	50	<0.0001	1,14	159	NA	2,26	2295	NA
C/N	2,25	33	<0.0001	1,14	1.3	NA	2,25	456	NA
Soil C_F[b]	2,25	0.1	0.95	1,14	2.0	0.18	2,25	173	<0.0001

NA=not applicable; *P*-values are excluded due to significant interaction with other variable.
[a] *df*=degrees of freedom.
[b] Soil C_F=the percentage of total soil C found in fractions.

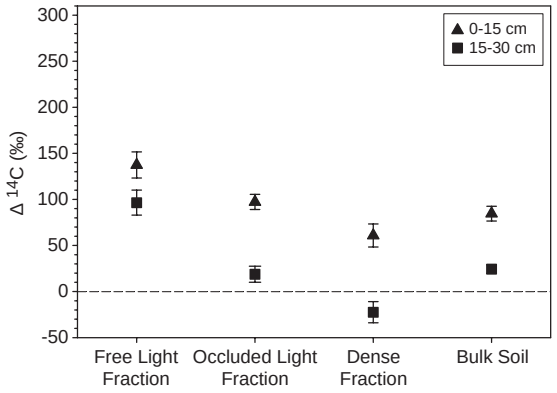

Fig. 2. $\Delta^{14}C$ (‰) of density fractions and bulk soil from 0–15 cm and 15–30 cm at Walker Branch. Error bars are 1 SE.

recovered, and was probably lost as dissolved organic C (NaPT- and water-extractable) during rinsing of the fractions. Comparable C loss from some soils was also reported by Golchin et al. (1994a,b).

The C concentrations of the fractions changed differently with depth, resulting in a statistical interaction (Table 2). The C concentration of the DF decreased by nearly five times with depth, the free LF increased slightly, and the occluded LF showed no change. Thus, although the occluded LF had higher C concentrations than the free LF in the upper soil (t_{25}=4.48, P=0.0001), the evidence for a difference was weaker in the lower soil increment (t_{25}=1.94, P=0.06). The N concentrations of the fractions also changed differently with depth, resulting in a statistical interaction (Table 2). There was no statistical evidence of a difference between the N content of the free and occluded light fractions, and the LF N concentrations were at least ten times higher than those of the DF. The N by depth interaction appeared to result from a steeper decrease in N concentration in the DF with depth than in the light fractions.

The C/N ratios of the light fractions and the DF showed opposite trends with increasing depth, resulting in a significant interaction (Table 2). The C/N of

Table 4
Analysis of variance (ANOVA) results for main effects and interactions of soil variables at TVA arranged and tested according to split-plot designs

Variable	Depth×Fraction			Depth[a]		Fraction[a]	
	df[b]	*F*	P	*df*[b]	*F*	*df*[b]	*F*
$\Delta^{14}C$	2,26	4.3	0.0245	1,13	287	2,26	307
C	2,26	61.4	<0.0001	1,13	0.01	2,26	11492
N	2,26	30.3	<0.0001	1,13	73	2,26	2170
C/N	2,26	52.9	<0.0001	1,13	6.4	2,26	53
Soil C_F[c]	2,26	10.5	0.0006	1,13	0.52	2,26	895

[a] *P*-values are excluded due to significant interaction with other variable.
[b] *df*=degrees of freedom.
[c] Soil C_F=the percentage of total soil C found in fractions.

Table 3
Mass recovery, C and N concentrations, and distribution of soil C in density fractions in the 0–15 cm and 15–30 cm soil increments of TVA

	Mass (% of bulk soil)	C[a] (g kg^{-1})	N[a] (g kg^{-1})	C/N	Distribution of C (% of bulk soil)[b]
0–15 cm					
Bulk soil	NA	24.87±0.72	1.18±0.05	21.2±0.6	NA
Free LF	2.16±0.10	295.64±5.03	10.29±0.36	28.9±0.8	25.58±0.82
Occl LF	0.88±0.06	376.18±11.14	8.54±0.44	44.6±2.0	13.33±1.03
DF	96.96±0.08	13.33±0.45	0.88±0.04	15.2±0.3	51.92±1.10
Total recovery	99.84±0.14	NA	NA	NA	90.46±1.33
15–30 cm					
Bulk soil	NA	8.50±0.55	0.42±0.03	20.4±1.1	NA
Free LF	0.50±0.04	318.27±2.68	8.63±0.30	37.1±1.3	19.27±0.87
Occl LF	0.32±0.03	410.42±12.85	7.60±0.35	54.4±1.8	15.59±0.76
DF	99.18±0.06	4.58±0.35	0.40±0.02	11.4±0.5	54.71±1.03
Total recovery	99.36±0.20	NA	NA	NA	89.57±1.39

Values are ±1 SE.
NA=not applicable.
[a] g kg^{-1} of fraction.
[b] Percentage of total soil C found in a given fraction.

the DF decreased with depth whereas the C/N ratios of both light fractions increased with depth.

The $\Delta^{14}C$ of the density fractions interacted significantly with soil increment (Table 2), in that the $\Delta^{14}C$ of the protected-C fractions (occluded LF and DF) decreased more with depth than the free LF (Fig. 2). Within this trend, the free LF had greater $\Delta^{14}C$ than the occluded LF, which in turn was higher than the DF.

3.2. TVA (Enriched site)

Specific mass recoveries, C and N concentrations, and soil C distribution, are located in Table 3. The C and N values at TVA followed the same patterns as those at Walker Branch, including disproportionately high soil C in the light fractions and the highest concentration of C and widest C/N ratio in the occluded LF. However, there were some exceptions. Specifically, there was a significant interaction between the distribution of soil C in the fractions and soil increment (Table 4), which reflected lower C content in the 15–30 cm free LF as compared to the surface soil, and weak evidence for a higher C content in the occluded LF (t_{26}=1.72, P=0.09) and DF (t_{26}=1.83, P=0.08) at depth. The free LF contained higher N concentrations than the occluded LF in both depths, although the DF had a much lower N concentration that decreased more with depth than either light fraction.

The $\Delta^{14}C$ of the density fractions interacted significantly with soil increment (Table 4), but apparently not in the same manner as the $\Delta^{14}C$ in the fractions from Walker Branch (Figs. 2 and 3). As with the Walker Branch fractions, the free LF exhibited the highest ^{14}C activity of the three fractions. However, at 0–15 cm the $\Delta^{14}C$ in the DF was higher than in the occluded LF, and not different in the 15–30 cm soil increment.

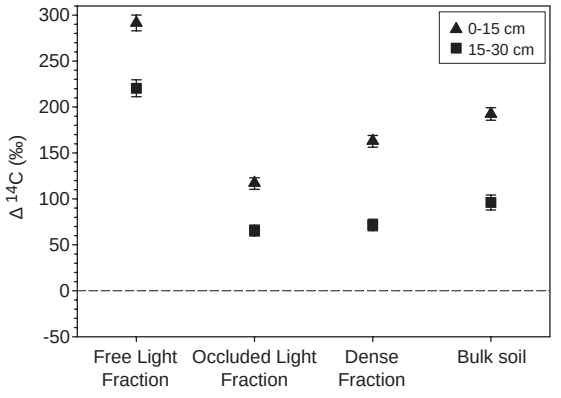

Fig. 3. $\Delta^{14}C$ (‰) of density fractions and bulk soil from 0–15 cm and 15–30 cm at TVA. Error bars are 1 SE.

4. Discussion

Our results show that the three-pool density fractionation procedure (Golchin et al., 1994a) separates SOM in these soils into three fractions with different physical and chemical characteristics, activity, and, presumably, C stabilization mechanisms. These fractions are the particulate organic matter (POM) found outside of aggregates (free LF), POM found within aggregates (occluded LF), and a mineral-associated carbon fraction (DF). One quarter of the soil C in each depth was free LF, and about 65% was stabilized by occlusion or organo-mineral interactions. There are several lines of evidence that there are different processes of stabilization, or cycling, among these fractions, including %C, C/N, ^{14}C, and links to ^{13}C NMR from other studies.

At the most basic level, the C concentrations and C:N ratios of the fractions indicate that they are distinctly different. The DF was 1% C, whereas the occluded and free LF were 40% and 25%, respectively. These values are comparable to those obtained by Golchin et al. (1994a) and are typical of the disproportionate soil C in density fractions (Dalal and Mayer, 1986; Christensen, 1992; Cromack et al., 1999; Parker et al., 2002). Analysis of the light fractions from Walker Branch and TVA soils support the characterization of the occluded LF as a more protected, less active fraction than the free LF (Golchin et al., 1994a; Baldock et al., 1997). The observation that C concentration and C/N ratios were higher in the occluded LF than the free LF is consistent with the findings of Golchin et al. (1994a), who found that the occluded LF contained higher C concentrations and comparable or higher C/N ratios than the free LF from several forest and grassland soils in Australia. Parker et al. (2002) also reported higher C concentration and C/N ratios in the occluded LF from several forest soils in New

England. In extensive ^{13}C NMR characterizations, the occluded LF contained lower proportions of *O*-alkyl-C, higher proportions of alkyl-C, and higher aromaticity (Golchin et al., 1994a; Sohi et al., 2001) compared to free LF, which indicates that the occluded fraction is more degraded and recalcitrant (Baldock et al., 1992; Baldock et al., 1997) or contains black C (Baisden et al., 2002b).

Trends in $\Delta^{14}C$ between fractions and depths support the basic conclusions from the C concentrations and C/N ratios. We can use the ^{14}C signatures to compare relative residence times among the fractions and between depths. Translating the $\Delta^{14}C$ to precise turnover times is problematic because of the combination of bomb spike and local pulse. The two clearest trends were the consistently high $\Delta^{14}C$ of the free LF, and the consistent decrease in $\Delta^{14}C$ with depth (Figs. 2 and 3). The high $\Delta^{14}C$ of the free LF's confirms that the free LF is the active fraction, most responsive to changes in C input (Dalal and Mayer, 1986; Janzen et al., 1992; Alvarez and Alvarez, 2000; Compton and Boone, 2000). In the Walker Branch fractions, $\Delta^{14}C$ decreased across fractions with increasing mineral association. The decrease in SOM $\Delta^{14}C$ with depth has been reported by numerous studies (Trumbore et al., 1989; Torn et al., 1997; Gaudinski et al., 2000; Paul et al., 2001; Baisden et al., 2002a; Kaiser et al., 2002; Rumpel et al., 2002; Certini et al., 2003), and is typically associated with C stabilization. Kaiser et al. (2002) measured increasing proportions of HF-acid-soluble organic C, in addition to decreasing $\Delta^{14}C$ with depth, and concluded that SOM at greater depths has increased mineral association and protection. Rumpel et al. (2002) found that soil C age and alkyl-C increased with depth, which they attributed to chemical and physical stabilization. We found that the difference in $\Delta^{14}C$ between the unprotected (free LF) and protected (occluded LF and DF) fractions increased with depth, indicating increased efficiency of mineral protection with depth.

The fractions from TVA require a more detailed interpretation, as the ecosystem had prolonged exposure to elevated atmospheric ^{14}C (Figs. 1 and 3). In the 10 years prior to the large ^{14}C pulse in 1999, ambient ^{14}C levels decreased at Walker Branch, from about 150‰ in 1989 to about 100‰ in 1999 (in tree ring cellulose). In contrast, however, tree core ^{14}C was higher each year at TVA after 1995, and in 1999 was nearly 400‰. The elevated ^{14}C was incorporated into the free LF at TVA, but was also apparent in the DF. The response of the free LF was expected, because it quickly responds to C inputs, and the photosynthetic products at TVA were elevated in ^{14}C for several years. However, the large incorporation of the label into the DF at TVA does not fit the conceptual model of the DF as a stable, passive C pool. The values of the DF at TVA sharply contrast those at the near-background Walker Branch, which had a depleted ^{14}C signature relative to the free and occluded LF (indicating slower turnover). Rapid response by the DF to inputs has been observed in other studies, though. For example, increases in litter inputs resulted in increases in the amount of DF in a temperate forest soil (Boone, 1994). Using acid hydrolysis, Trumbore et al. (1989) and Trumbore and Zheng (1996) separated the DF into hydrolysable (rapid) and non-hydrolysable (passive) pools that had different ^{14}C values.

The DF in these soils appears to contain at least two different C pools: an older, more stable pool of C, and a more recent, fast-cycling C pool. To the extent that the DF acts as a sink for microbial byproducts and a medium for microbial biomass (Chotte et al., 1998), it must include a C component that can cycle rapidly (Trumbore et al., 1989; Boone, 1994; Golchin et al., 1996; Trumbore and Zheng, 1996). Swanston et al. (2002) incubated the LF and DF from forest soils separately, and found no difference in the rate of respiration for the first 120 days. Therefore there must be some labile material associated with the dense fraction. However, different patterns of net N mineralization, indicated that the chemistry of the labile C was not the same in the two fractions (Swanston et al., 2004). It seems likely that the ^{14}C of the DF at TVA was enriched by recent plant inputs and associated microbial byproducts. This fast-cycling pool must be closely associated with the DF, considering that it withstood mixing and sonication in NaPT and repeated rinsing in water.

Golchin et al. (1994b), based on adding two intermediate densities to their earlier method (Golchin et al., 1994a), proposed a conceptual model that could explain the incorporation of recent SOM into the DF. Stable aggregates form as free LF is colonized by microbiota and encrusted with mineral particles, which are stabilized by microbial exudates. High-

quality organics such as proteins and carbohydrates are degraded and converted to microbial biomass and exudates, selectively preserving more recalcitrant alkyl and lignaceous compounds in the organic core of the aggregate. As the organic substrate becomes increasingly recalcitrant, the microbial community declines and the aggregate becomes unstable. In the later stages of the cohesive aggregate, the conservation of recalcitrant molecules and decrease of microbial biomass and byproducts results in an increase in the C concentration and C/N ratio of the remaining organic core. At these stages, the biomass and exudates are more associated with the mineral matrix of the aggregate (i.e., the DF), decreasing its C/N ratio. A key aspect of this conceptual model is that more recently-formed aggregates with carbohydrate-rich cores are resistant to ultrasonic disruption and ultimately separate into one of the intermediate densities (1.80–2.0 g mL^{-1}), which corresponds to part of the DF in the present study. Thus, if the combination of physical and ultrasonic disruption we applied to the soil did not completely disperse recently-formed micro-aggregates, some of these micro-aggregates may have contained elevated ^{14}C and ultimately separated into the DF. This is essentially what we observed: high incorporation of the recent pulse-^{14}C into the free LF, concurrent with or followed closely by the elevated ^{14}C in the DF. The occluded LF appeared to incorporate little of the ^{14}C pulse. If the model continues to accurately predict processes in these soils in subsequent years, the elevated soils at TVA should show a dilution of the elevated $\Delta^{14}C$ in the free LF and DF, and a concomitant increase in the $\Delta^{14}C$ in the occluded LF.

Baisden et al. (2002b) proposed an alternate conceptual model to explain natural abundance ^{15}N and ^{14}C concentrations in density fractions separated similar to those of Golchin et al. (1994b) from several grassland soils in the Central Valley of California. Namely, that the free LF is humified and gradually converted to occluded LF as carboxylic groups both increase and bind with clay. To the extent that microbial byproducts are stabilized during the gradual process of occlusion and mineral association, the C/N ratio of the protected fractions could be expected to be lower than the initial plant-like value. Continued degradation of the occluded fraction is negligible until the aggregate is physically disturbed. Considering the results from TVA within the context of the Baisden model, as the elevated ^{14}C is removed from the free LF through degradation or occlusion within aggregates, the ^{14}C concentrations in the occluded LF and the DF should rise concurrently. Indeed, both fractions appeared to incorporate some of the elevated ^{14}C, but the incorporation was heavily weighted to the DF. These initial results appear to be more clearly explained and predicted by the Golchin conceptual model.

5. Concluding remarks

We have shown that the three-pool density fractionation introduced by Golchin et al. (1994a), using density and increasing aggregate disruption to fractionate soil, has been successful in isolating meaningful soil organic and organo-mineral fractions from upland Ultisols at Oak Ridge Reservation, Tennessee. These fractions (free LF, occluded LF, and DF) differ in fundamental qualities such as C concentration, C/N ratio, $\Delta^{14}C$, change with soil depth, and the timing and magnitude of response to inputs of elevated ^{14}C. In these soils the free LF (inter-aggregate POM) is clearly the most responsive and least protected organic fraction. The occluded LF (intra-aggregate POM) is more isolated from recent plant inputs than the DF (mineral-associated C), although this does not mean that occlusion within these aggregates provides more C stability than organo-mineral interactions. On the contrary, the ^{14}C values from Walker Branch and TVA suggest that whereas the DF quickly incorporates plant inputs, it also contains a much older and more stable C pool than does the occluded fraction. The increase in the difference in $\Delta^{14}C$ between unprotected and protected SOM with depth suggests that the importance of physical and organo-mineral C stabilization processes, compared to the importance of rates of C input from plants, increases from the surface to deep soil.

In subsequent EBIS work we will use these methods to trace the movement and stabilization of ^{14}C from roots and litter using reciprocal litter transplants across the ^{14}C-enrichment gradient. The combination of the ecosystem ^{14}C enrichment gradient, the litter transplants, and the density fractionation, should give us insight into the specific roles of root versus litter inputs into these stabilization process.

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