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Soil organic matter is principally root derived in an Ultisol under oak forest

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ABSTRACT

To a large degree, the sources and stability of soil organic carbon remain poorly constrained. A clear understanding of links among the components of the soil C cycle is hampered by the complexity of the system as well as challenges associated with partitioning bulk soil C into meaningful fractions. A large accidental $^{14}CO_2$ release at the Oak Ridge Reservation in Tennessee, USA provided a strong label pulse into adjacent, well-studied oak forests, resulting in highly elevated $\Delta^{14}C$ values in leaf litter (~1000‰) and roots (~260–450‰). A four-year manipulative study was conducted to determine the relative contribution of litter versus roots to the bulk mineral soil C pool, as well as to free light, occluded light and heavy fractions. The heavy fraction was further split into fractions with densities of 1.7–2.4 g cm^{-3} and >2.4 g cm^{-3} to test the homogeneity of the mineral-associated fraction of C. Substantial concentrations of label were detected in all soil fractions within a year of the $^{14}CO_2$ release, indicating rapid incorporation of newly fixed photosynthates in all fractions of soil organic C, regardless of differences in stability inferred by previous work. This rapid incorporation of label occurred only in treatments where roots were labeled, indicating that roots are the major source of inputs to mineral soil C stocks at these sites. Separation of the heavy fraction into subfractions of intermediate (1.7–2.4 g cm^{-3}) and high (>2.4 g cm^{-3}) density indicated that both subfractions incorporated label at similar rates, despite significant differences in degree of microbial processing. In general, the rate of label incorporation suggested a much faster turnover for all fractions than indicated by natural radiocarbon abundance values. This suggests that within each soil fraction there are portions of slow-cycling and fast-cycling materials, and the determination of an average turnover time or mean age is dependent on experimental approach. The rapid incorporation of label into all fractions within a year regardless of inferred stability implies a high degree of heterogeneity in all fractions regardless of how finely the soils are partitioned. Further refinement of the nature and drivers of this heterogeneity could yield important insights into the soil C cycle.

1. Introduction

Maintaining, and if possible, increasing soil organic C (SOC) stocks has garnered increasing interest as a potential climate change mitigation strategy (Griscom et al., 2017; Minasny et al., 2017; Paustian et al., 2016). However, our ability to accurately model and manage SOC stocks is hindered by a lack of clarity on some of the most basic processes regulating SOC stocks. For example, previous research has suggested that the relative importance of different C sources to the soil (e.g. roots versus litter) varies with ecosystem factors such as vegetation composition and soil properties, but the magnitude (and even the very direction) of these differences is not currently well defined (Bradford et al., 2016). Measurements across several adjacent forest vegetation types on loamy, high-activity Ultisols indicated litter inputs were consistently restricted to topsoils; root inputs varied with depth and dominant tree species and were the dominant source of SOM in subsoils (Spielvogel et al., 2016). In contrast, examination of coarse-textured Spodosols under two forest vegetation types indicated that dissolved organic C

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from litter accounted for 80–95% of total C inputs to subsoils, varying with dominant tree species (Rothstein et al., 2018). These differences in C source have implications for the quality and stability of resulting SOC stocks. Specifically, a *meta*-analysis of forest and grassland litterbag and incubation studies suggested that root-derived SOC persists in soils an average of 2.4 times longer than shoot-derived SOC (Rasse et al., 2005). Similarly, a subsequent study found 28% higher retention of introduced root material in comparison to needleleaf material in an incubation tracer experiment (Bird et al., 2008). The strong influence of C source on SOC persistence, in combination with the high variability of root versus litter inputs across vegetation and soil types suggests further investigation across a diversity of soils is needed.

Additionally, though many conceptual models of the SOC cycle have been proposed, the actual pathways of C movement and transfer among pools of differing stability have not been definitively established due to the large number of confounding and/or interacting processes and controls at work in any individual soil (Schmidt et al., 2011). Some models separate bulk SOC into subfractions or pools of differing stability, which may include fractions representing unprotected particulate organic matter (free/light fraction), particulate organic matter occluded within aggregates (occluded light fraction), and organic matter associated with mineral surfaces (heavy fraction) e.g.(Sierra et al., 2014). These fractions correspond to those which can be feasibly physically separated from one another in the lab using a combination of density fractionation and aggregate disruption techniques (Golchin et al., 1994; Swanston et al., 2005), and which align conceptually with known mechanisms of soil C stabilization (e.g. (von Lützow et al., 2007). Historically, models suggested that new C inputs to soils first enter the free/light fraction then move through the occluded light fraction and are subsequently stabilized in the heavy fraction, e.g. (Golchin et al., 1994; Wagai et al., 2009). Though these pathways are generally supported by observational studies, how they may vary across ecosystems and soils is not known. Additionally, the role of the microbial community in the production and stabilization of SOM is currently undefined. Recent work has suggested that microbial necromass may account for as much as half of SOC (Liang et al., 2019), a finding generally supported by the microbial nature of organics associated with mineral surfaces (Jastrow et al., 1996). However, the relative importance of litter versus roots as a source of microbial substrate is not clearly defined in most C cycle models.

One approach to elucidating the soil C cycle is through the addition of a reactive tracer, which allows the researcher to track movement of the tracer through the system over time but does not alter the soil C cycle's function. Radiocarbon (^{14}C) has been a commonly used tracer across a myriad of systems and scales since the bomb spike was discovered in the 1960′s (Cook et al., 2009; Harkness, 1970), and its utility with respect to understanding C cycle dynamics in the pedosphere was soon leveraged in a variety of soil C studies (Harkness et al., 1986; O'brien and Stout, 1978). However, the spike is limited in both magnitude and time scale. The bomb spike roughly doubled the amount of radiocarbon in the atmosphere by 1967, but afterwards the spike rapidly declined, making clear detection of the uptake of the bomb spike among pools and with depth difficult to detect in many soils. Additionally, multiple time points are required to estimate transfer rates and patterns, necessitating multiple sampling events many years apart, or the existence of archived samples.

The Enriched Background Isotope Study (EBIS) on the Oak Ridge Reservation in Tennessee, USA offered a unique opportunity to utilize a large and accidental release of ^{14}C radiocarbon tracer. In 1999, a large release of ^{14}C-enriched CO_2 from a local incinerator resulted in labeling of the surrounding forests (Trumbore et al., 2002), creating an extremely rare whole system tracer experiment. Following discovery of the event, researchers launched a series of investigations into the terrestrial C cycle including examination of soil respiration, dissolved C, microbial biomass, forest floor, roots, mycorrhizae, and mineral soils (Kramer et al., 2010; Swanston et al., 2005; Tipping et al., 2012; Treseder et al., 2006), building upon existing understanding of C cycling at this long-term ecosystem study site (Curtis et al., 2002; Davidson et al., 2002; Hanson et al., 1993; Johnson and Van Hook, 2012). This labeling event was similar in magnitude to the bomb spike, but highly localized. Early detection of the labeling event allowed for the installation of litter-swap treatments between labeled and unlabeled sites. This litter-swap manipulation allowed for comparison of the relative importance of roots versus leaf litter as a source of C to soils on a yearly basis. We used measurements of radiocarbon abundance of organic horizons and mineral soils over a period of four years to gain insight into the sources, pathways, and stability of distinct pools of soil C with a specific focus on the following questions:1) In this temperate forest ecosystem, what is the largest source of C to soil: litter or roots? 2) Are there significant differences in annual C partitioning among different pools/fractions of SOC? 3) Specifically, how is tracer transferred among fractions? 4) How quickly does newly introduced C propagate to depth? and, 5) How homogeneous are C uptake rates within the mineral-associated pool/fraction of SOC?

2. Methods

2.1. Site and experiment description

In 2001, sixteen 7x7 m experimental plots were established at two sites on the Oak Ridge Reservation: Tennessee Valley Authority and Walker Branch (Swanston et al., 2005; Trumbore et al., 2002) (Fig. 1, Supp. Fig. 1). The sites are very similar in all ecosystem properties, including topography (upper slope and ridge positions), parent material (dolomitic and cherty limestone residuum), vegetation (dominantly *Quercus* spp. and *Acer rubrum*., with *Carya* spp., *Pinus echinata*, *P. virginiana*, *Liriodendron tulipifera*, *Fagus grandifolia* and other accessory taxa) and soils (Fullerton series Typic Paleudults (USDA classification); Haplic Alisol (WRB classification)) with clay phases dominated by kaolinite and hydroxy-Al interlayered vermiculite. Sand/silt/clay percentages ranged from 36/62/2 in the uppermost surface layer to 12/49/39 in the deepest layer. No carbonates were present in the soils, as pH values ranged from 5.0 in the surface to 3.9 at depth (Peters et al., 1970).

In 1999, the Tennessee Valley Authority site was exposed to very high levels of $^{14}CO_2$, and thereby the trees at this site incorporated a large amount of ^{14}C label. Because the trees at Tennessee Valley Authority incorporated high levels of $^{14}CO_2$ into their photosynthates, the leaf litter and roots they produced were also highly labeled. The enriched litter at Tennessee Valley Authority $\Delta^{14}C$ values were ~ 1000‰, while the enriched roots varied from 261 to 455‰ (Fröberg et al., 2007; Joslin et al., 2006). Walker Branch was exposed to a much lower and shorter pulse of $^{14}CO_2$. Therefore, even though tree rings show some incorporation of ^{14}C label at this site, levels are far below that of Tennessee Valley Authority, and the litter and roots they produced were near ambient atmospheric values at that time (litter ~221‰, roots ~125–294‰). Employing a leaf litter replacement approach between the two sites, a total of four treatments were established across the two sites with four replicates each, yielding sixteen experimental plots total. Litter for the multi-year litter replacement study was collected in the fall of 2000 on Walker Branch (ambient litter) and Pine Ridge across the valley from the Tennessee Valley Authority site (enriched litter). Enough litter was collected in 2000 to provide material for three years of subsequent treatment applications. During litter replacements over the following three years (March of 2001, January/February 2002 and 2003) approximately 500 g dry matter m^{-2} of the respective litter types were added to each of the plots, corresponding to the average annual litterfall at the sites. Each fall, natural litterfall for each plot fell onto a 7x7 m section of landscape cloth allowing for its removal and replacement by the target litter types. At Walker Branch, the treatments included control plots with ambient litter inputs (*control treatment;* replacement ambient leaf litter and natural root turnover) and enriched foliar litter plots with natural root turnover

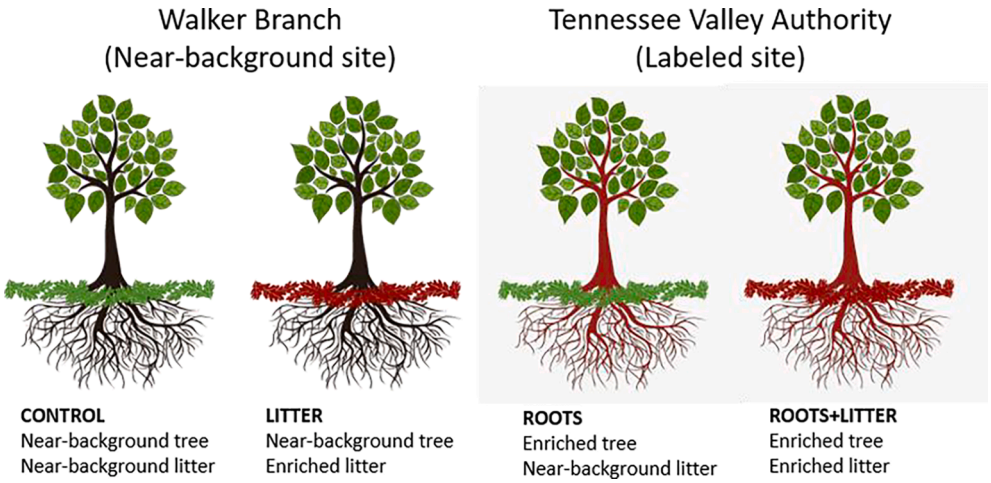

Fig. 1. Illustration of four treatments in the current study. The treatments are named according to the primary source of radiocarbon label in the study (labeled source in red). Please see Supplementary Fig. 1 for a more comprehensive illustration of sampling design.

(*litter treatment*). At Tennessee Valley Authority, the treatments included a roots only enriched plots with ambient foliar litter (*roots treatment)*, and enriched foliar litter and root input (*roots + litter treatment)* (Supplementary Fig. 1).

It is important to note that the application of labeled and near-background litter at the *litter* and *roots* treatment plots was instigated in March of 2001, approximately one and a half years after the release of the $^{14}CO_2$ plume at Tennessee Valley Authority which occurred between July and August of 1999 (Trumbore et al., 2002). From the time of the $^{14}CO_2$ release in 1999, ^{14}C label was actively incorporated into root material at Tennessee Valley Authority though leaves did not show evidence of strong incorporation of the label until formation of new buds in spring of 2000 (Trumbore et al., 2002). Therefore, introduction of ^{14}C label from root exudates and turnover at Tennessee Valley Authority was occurring 1–2 years prior to possible introduction of ^{14}C label from applied leaf litter at the *litter* and *roots + litter* treatments. Analysis of tree rings also indicated additional lower-level unquantified enrichment events over time at Tennessee Valley Authority (Trumbore et al., 2002). Those events increased the treatment effect at Tennessee Valley Authority, especially those associated with root inputs.

2.2. Soil and litter sample collection

Soil and litter samples were collected on the Oak Ridge Reservation from each of the 16 experimental plots at Walker Branch and Tennessee Valley Authority sites in March 2001, January 2002, January 2003, and January 2004. Soil was collected from organic horizons (Oi material older than one year and Oe/Oa) to the surface of the mineral soils using a 17.84 cm diameter ring (0.25 m^2 area). Mineral soils from 0 to 15 cm, 15–30 cm, 30–60 cm, and 60–90 cm were collected with a 10.2 cm diameter core sampler (one sample per plot per year). These depths were chosen in order to capture differences in label incorporation with depth. Fifteen cm increments were sampled near the surface in the more actively cycling soil layers. Larger depth increments (30 cm) were sampled in more C-poor deep soil layers. Soils were stored at −20 °C until further processing. After thawing, soils were sieved to < 2 mm, root picked, and oven dried at 105 °C.

2.3. Soil analysis

Soils from the 0–15 cm and 15–30 cm depths were density separated into free light fractions (FLF), occluded light fractions (OLF) and heavy fractions (HF) using a sodium polytungstate solution adjusted to a density of 1.70 g cm^{-3} and sonication to disrupt aggregates (200 J mL^{-1}) (Golchin et al., 1994; Swanston et al., 2005). Samples were ground prior to further analysis. A selected subset of heavy fractions from 2004 was further density separated into a fraction with a density range of 1.7–2.4 g cm^{-3} and a fraction with a density > 2.4 g cm^{-3}, with the goal of investigating the level of homogeneity in incorporation of label in the heavy fraction. A separation at 2.4 g cm^{-3} was chosen in an effort to separate the heavy fraction into a fraction rich in phyllosilicate clays and a denser fraction rich in quartz. Only samples from 2004 from the *control, litter,* and *roots* treatments were density separated into 1.7–2.4 and > 2.4 cm^{-3} fractions. Due to the time and expense associated with these additional density separations, only a small subset of the heavy fractions could be examined. Samples from the 2004 were chosen since these samples represented the longest exposure time since labeling and therefore were hypothesized to show the largest treatment effect.

Total nitrogen (N) and C concentrations, and stable C and N isotope analyses of bulk soils and soil fractions were performed at Lawrence Berkeley National Laboratory. Samples were ground, powdered, and aliquots were loaded in Sn capsules for analysis. The capsules were then loaded into a zero blank autosampler connected to an ECS 4010 Elemental Analyzer (Costech Analytical Technologies Inc., Valencia, USA) coupled to a Delta V^{plus} isotope ratio mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). Based on laboratory internal standards, the analytical precision on concentrations are ± 0.05 % weight (1σ) for nitrogen, ± 0.59 % weight (1 σ, where σ is standard deviation) for carbon, for $\delta^{13}C_{VPDB}$ ± 0.21‰ (1 σ), and for $\delta^{15}N_{AIR}$ ± 0.32‰ (1 σ).

Samples were graphitized in preparation for ^{14}C abundance measurement at the Center for Accelerator Mass Spectrometry at Lawrence Livermore National Laboratory. Following sieving, samples were dried, weighed into quartz tubes along with silver and cupric oxide, and sealed under vacuum. Samples were combusted at 900 °C for 6 h to form CO_2 gas. The CO_2 was then reduced to graphite through heating at 570 °C in the presence of hydrogen (H_2) gas and an iron (Fe) catalyst (Vogel et al., 1987). Graphite targets were then analyzed for radiocarbon abundance (Davis et al., 1990), and corrected for mass-dependent fractionation following (Stuiver and Polach, 1977). Radiocarbon measurements were conducted on all free light, occluded light, and heavy fractions, bulk soils, as well as the 1.7–2.4 and > 2.4 fractions.

2.4. Statistical approach

Our statistical approach tested for the effect of treatment (root versus litter) over time, with depth, and among density fractions. Specifically, we looked for evidence of uptake of label from either roots or litter to the surrounding soil. Due to differences in length and degree of exposure to the labeling event, Walker Branch and Tennessee Valley Authority were

analyzed separately. Variance in radiocarbon abundance values ($\Delta^{14}C$) in fractions within each site was assessed using the GLIMMIX procedure and a repeated measures split-split-plot design with a normal distribution and an identity link function using SAS 9.4 (Cary, 2011). Treatment, depth, year, fraction and their interaction terms were tested for significance as fixed effects. Plot, plot*average depth, and plot*average depth*fraction were the random effects in the model. To assess the correlation between years, an auto-regressive order 1 covariance structure was used, and a Kenward Rogers denominator degrees of freedom adjustment was applied. Residuals were evaluated for homogeneity of variance and normality and were adjusted to include a heterogeneous covariance structure or using the group = option when needed. Tukey-Kramer Least Squares Means Adjustment for Multiple Comparisons was used to test for significant differences among treatments, depths, years, fractions, and their associated interactions for significant fixed effects. Variances in bulk $\Delta^{14}C$ and fraction $\Delta^{14}C$ values were assessed in two separate sets of models. One set included data associated with the free light, occluded light, and heavy fractions, the other set included only the bulk soil values. Variance in radiocarbon abundance values ($\Delta^{14}C$) in bulk soils within each site was modelled under the same conditions as the fractions, but with a repeated measures split-plot design. Due to the limited size of the sample set, 1.7–2.4 and > 2.4 g cm^{-3} fractions were only compared using a series of paired *t*-tests. Pearson correlation coefficients of a linear relationship were calculated to assess the presence and strength of associations among free light, occluded light and heavy fractions, and bulk soils.

2.5. Estimation of turnover times

We estimated the turnover time of organic C from the 0–15 cm layer of soil at the ORR in two ways. First, we used radiocarbon data from density fractionations of soils from the ambient treatment of the Throughfall Displacement Experiment which was carried out in plots adjacent to the current experiment (Hanson et al., 2003). Soils were sampled prior to the $^{14}CO_2$ labeling event, allowing for the estimation of a steady state mean residence time based on natural abundance radiocarbon measurements (Torn et al., 2002; Trumbore, 1993). Second, we examined the average rate of incorporation of the $^{14}CO_2$ label in the soils from 0 to 15 cm at the *roots* treatment plots. Because we did not have soil samples from the *roots* treatment plots prior to the labeling event, we compared the average radiocarbon values of the soils at the *roots* treatment in 2001 to the average radiocarbon values of the soils at the *control* treatment in 2001. We used a two-pool mixing model to assess the amount of new inputs into each fraction over a one-and-a-half-year period (i.e. label event happened in August 1999, soils were sampled in March 2001).

$$D_c P_c + D_r P_r = D_f$$

Where D_c is the $\Delta^{14}C$ of the *control* treatment as sampled in 2001, P_c is the fraction percentage of the sample's C with the $\Delta^{14}C$ of the *control* treatment, D_r is the $\Delta^{14}C$ of the labeled root material at the *roots* treatment, P_r is the fraction percentage of the sample's C with the $\Delta^{14}C$ of the labeled root material, and D_f is the $\Delta^{14}C$ of the density fraction from the *roots* treatment as sampled in 2001.

3. Results

Results are reported according to label and litter swap treatment. The near-background *control* and *litter* treatments were located at Walker Branch. The *control* treatment received no labeled litter and was not exposed to the large ^{14}C plume. Labeled litter was applied at the *litter* treatment. At the Tennessee Valley Authority Site, trees were labeled by the ^{14}C plume at the *roots* and *roots* + *litter* treatments. The *roots* + *litter* treatment additionally received labeled litter, whereas near-background litter was applied at the *roots* treatment.

3.1. Mass, C and N recovery

Mass recovery following density fractionation averaged 99.4 ± 0.3% at the Tennessee Valley Authority site, and 100.0 ± 0.1% at the Walker Branch site. Recovery of C following density fractionation was more variable. At Tennessee Valley Authority, C recovery averaged 93.0 ± 1.0%, with a maximum of 170% and a minimum of 58%. Walker Branch C recovery averaged 101.1 ± 2.6, with a maximum of 302% and a minimum of 71%. This variability in C recovery is not uncommon given the inherent heterogeneity in particulate organic matter distribution in soils and the variance associated with soluble C being lost during density separation (Crow et al., 2007). Variability in N recovery was very high, despite mass recovery values near 100%. This variability may be due to a combination of the low N concentrations of the soils and the low (in comparison to N concentrations) precision of the elemental analyzer. Following density separation into the 1.7–2.4 and > 2.4 cm^{-3} fractions, mass recovery ranged from 98 to 105% and averaged 100% of the starting heavy fraction mass. Recovery of C averaged 91% ± 7% (1 σ, σ = standard deviation) across both sites.

3.2. Bulk, free light fraction, occluded light fraction, heavy fraction: C and N contents, distribution, $\Delta^{14}C$ values

Percent total C values for bulk soils ranged from an average of 2.54% for surface soils (the 0–15 cm layer) to<1% for soils at depths of up to 1 m (Table 1, Supp. Fig. 2). Total N concentration of bulk soil ranged from an average of 0.12% to 0.01% (Table 1, Supp. Fig. 3). Percent C values for free light fractions averaged ~ 30%, occluded light averaged ~ 40% and heavy fractions ~ 1%. The majority of soil C resided in the heavy fraction (58%), with lesser amounts distributed to the free light (~30%) and occluded light (~13%) fractions (Fig. 2, Table 2, Supp. Fig. 4; note the high degree of variability in C distribution across time which is evident in all treatments). Percent N values for free light fractions averaged ~ 0.8%, occluded light averaged ~ 0.9% and heavy fractions ~ 0.05% (Supp. Fig. 5). When recalculated to sum to 100%, the majority of the N resided in the heavy fraction (83%), followed by the free/light fraction (12%), and the occluded/light fraction (5%) (Supp. Fig. 6).

Radiocarbon was measured on bulk mineral soils from four depths (0–15, 15–30, 30–60 and 60–90 cm), and three litter layers (foliar litter inputs applied each year, Oi older than one year and Oe/Oa) (Figs. 2 and 3). Measurements of the Oi layers in 2001 prior to installation of the litter swap treatment showed near-background litter at the *litter* treatment plots and enriched litter at the *roots* and *roots* + *litter* treatments. Measurements of Oi layers in subsequent years reflected the radiocarbon abundance of the litter applied to the plots. The $\Delta^{14}C$ values of the Oe/Oa layers appeared to gradually increase over time in the *litter* treatment and gradually decrease over time in the *roots* treatment but did not show large or immediate incorporation of the ^{14}C from the overlying Oi layer. In mineral soils, the treatments grouped by site, with bulk soils at Tennessee Valley Authority having elevated $\Delta^{14}C$ values in comparison to Walker Branch (Fig. 3, Table 3).

Only soils from the 0–15 and 15–30 cm depth increments were density separated and measured for $\Delta^{14}C$. In general, for all treatments and sites, $\Delta^{14}C$ values of the three primary density fractions followed the pattern free light > occluded light > heavy, and all fractions declined in $\Delta^{14}C$ value with increasing depth (Fig. 4). As was observed in bulk $\Delta^{14}C$ values, averaged fraction $\Delta^{14}C$ data also grouped by site, with Tennessee

Table 1
Means and standard deviations of bulk mineral soil C and N concentrations.

Depth (cm)	% C	±	% N	±
0–15	2.54	0.54	0.12	0.04
15–30	0.76	0.21	0.03	0.02
30–60	0.44	0.10	0.02	0.01
60–90	0.23	0.04	0.01	0.00

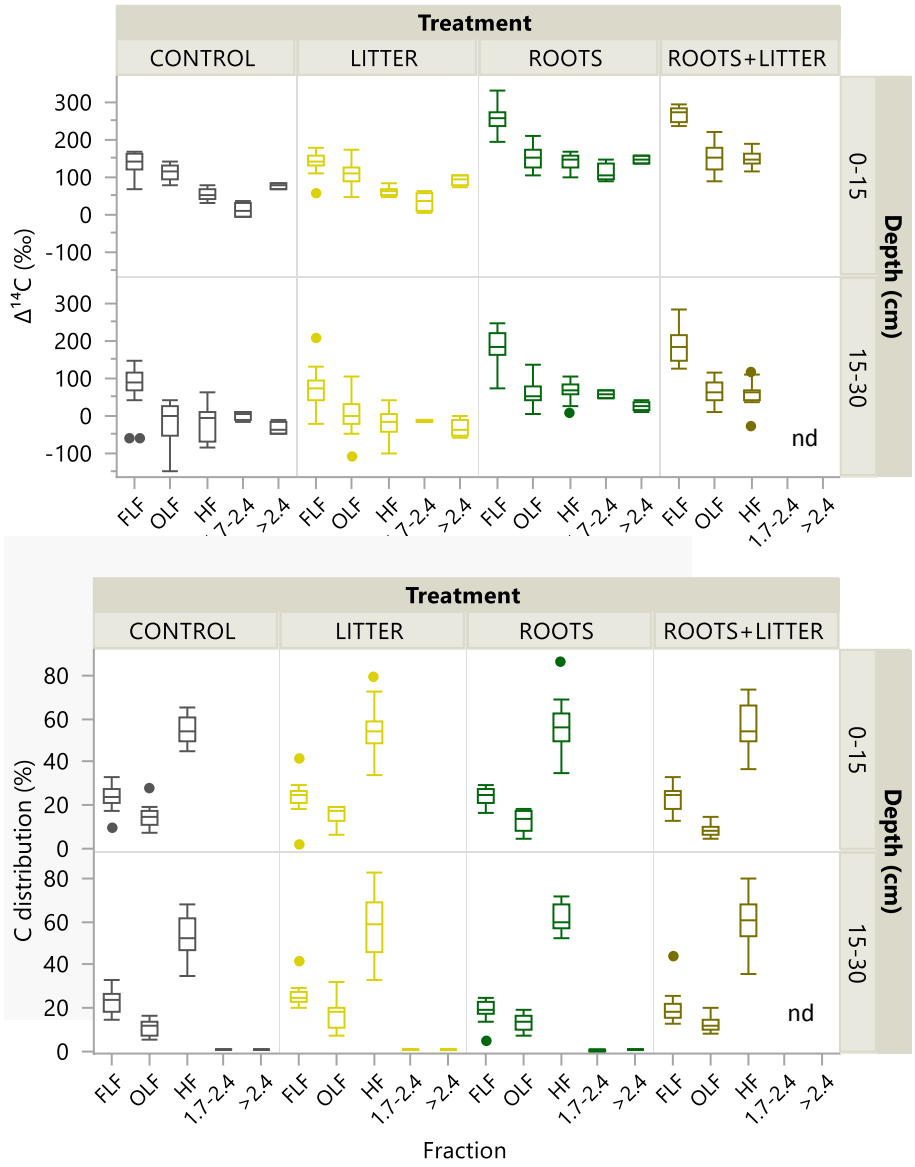

Fig. 2. Outlier box plots. Boxes represent the median, 1st and 3rd quartiles. Whiskers extend to 1.5x the interquartile range. Outliers are represented by individual data points outside the whisker range. Top: Δ ^{14}C (‰) values of density fractions, averaged across all four years of the study (2001–2004). Bottom: C distribution (%) among density fractions. FLF, HF and OLF sum to 100%. Values of the > 2.4 and 1.7–2.4 fractions sum to 100% of the corresponding HF C value. nd = no data.

Table 2
Means and standard deviations of free light, occluded light, and heavy fraction C and N concentrations.

Depth (cm)	Fraction	% C	±	C distribution (%)	±	% N	±
0–15	FLF	28.95	1.81	24	6	0.89	0.11
	OLF	41.62	5.24	12	5	1.00	0.16
	HF	1.43	0.27	56	10	0.08	0.02
15–30	FLF	32.99	2.82	22	6	0.72	0.10
	OLF	44.48	5.57	14	11	0.72	0.20
	HF	0.49	0.24	58	11	0.04	0.02

Valley Authority exhibiting elevated Δ ^{14}C values in comparison to Walker Branch (Table 4).

3.3. Effect of treatment and time in fractions

A comparison of the Δ ^{14}C values of the fractions at the two treatment installments at the Walker Branch site indicated that treatment was not a significant effect ($p = 0.5147$; Supp. Table 1). The Δ ^{14}C values of *control* and *litter* did vary as a function of depth ($p < 0.0001$), fraction ($p < 0.0001$), depth*fraction ($p < 0.0001$), year ($p = 0.0223$), year*depth ($p < 0.0001$), year*depth*fraction ($p = 0.0067$), and year*depth*-fraction*treatment ($p = 0.0395$).

A comparison of the Δ ^{14}C values of the free light, occluded and heavy fractions at the two treatment installments at the Tennessee Valley Authority site indicated that treatment was not a significant effect ($p = 0.8718$). The Δ ^{14}C values of *roots* and *roots + litter* treatments did vary as a function of depth ($p < 0.0001$), fraction ($p < 0.0001$), year*fraction ($p < 0.0001$), and year*depth ($p = 0.0247$). As evidenced by differences in p-values associated with individual fixed factors, in all cases, the significance of the interaction terms was driven by depth and fraction, with treatment and year having very little influence in the interaction. Across all years, fractions from 0 to 15 cm were significantly enriched in Δ ^{14}C in comparison to fractions from 15 to 30 cm of depth ($p < 0.0001$ for all comparisons). Across years, the free light fraction was always enriched in comparison to heavy and occluded light fractions ($p < 0.0001$ for all comparisons). Heavy and occluded light fractions varied in their

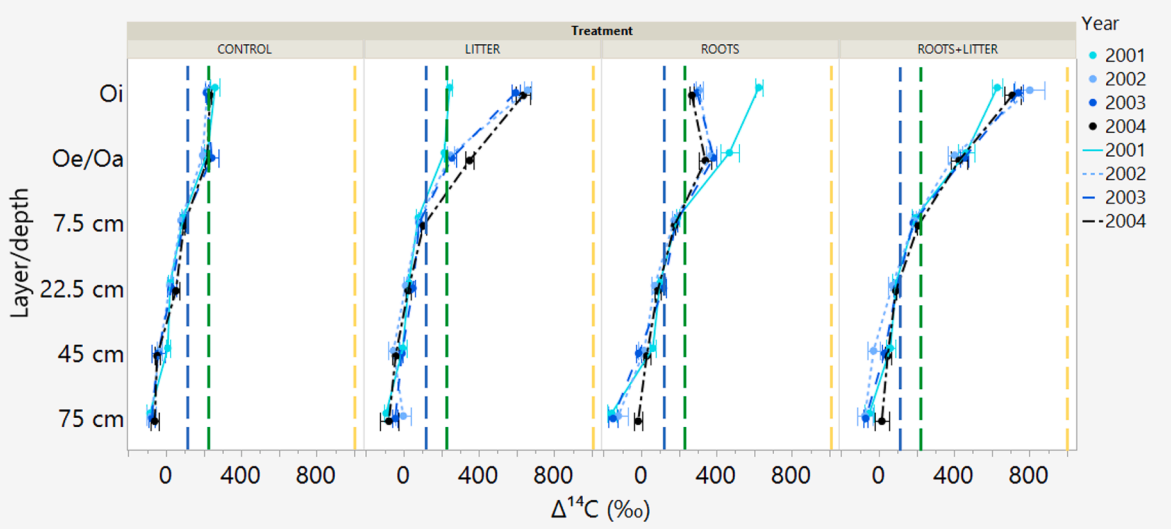

Fig. 3. $\Delta^{14}C$ values for bulk soils with depth over multiple years of litter replacement treatments. Droplines represent the $\Delta^{14}C$ values for the atmosphere in the year 2001 (blue), added enriched litter (yellow) and near background litter (green).

Table 3
Mean $\Delta^{14}C$ values of bulk mineral soils from 0 to 15, 15–30, 30–60 and 60–90 cm depths, associated standard deviations, and number of replicates (*n*) for treatments and sites.

Site	Treatment	*n*	Mean $\Delta^{14}C$	±
Walker Branch		118	7.5	68.9
	Control	60	3.5	71.5
	Litter	58	11.6	66.4
Tennessee Valley Authority		113	62.9	103.9
	Roots	59	61.2	110.2
	Roots + Litter	54	64.6	97.5

Table 4
Mean $\Delta^{14}C$ values of density fractions from 0 to 15 and 15–30 cm depths, associated standard deviations, and number of replicates (*n*) for treatments and sites.

Site	Treatment	*n*	Mean $\Delta^{14}C$	±
Walker Branch		192	56.0	73.6
	Control	96	56.4	71.2
	Litter	96	55.6	76.2
Tennessee Valley Authority		190	144.3	76.6
	Roots	94	142.7	74.9
	Roots + Litter	96	145.9	78.6

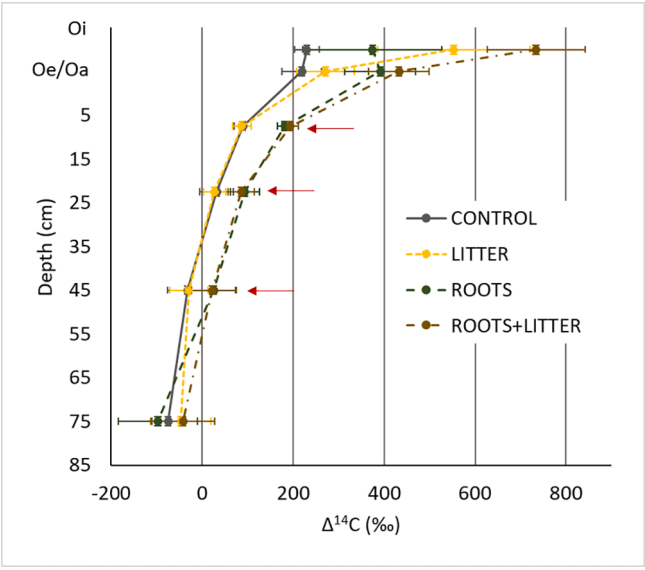

Fig. 4. $\Delta^{14}C$ values averaged over time (2001–2004) for bulk soils with depth. Note how treatments visually group by site, and the clear separation between Walker Branch treatments (*control, litter*) and Tennessee Valley treatments (*roots, roots + litter*) indicated by red arrows.

relationship over time. The four-way interaction term in the model comparing *control* and *litter* is most likely the product of increasing occluded light $\Delta^{14}C$ values in the *litter* treatment over time (Fig. 5). A summary of fixed effect statistics is given in supplementary Table 1.

Pearson correlation coefficients were calculated for pairwise comparisons of fractions and bulk soil $\Delta^{14}C$ values, shown as density ellipse fits (Fig. 6). Coefficients were interpreted as expressing either a strong ($r > 0.70$), moderate ($r = 0.50–0.69$), weak ($r = 0.30–0.49$), or very weak ($r < 0.29$) relationship between the two variables under comparison. Heavy and free light fractions were strongly related to each other and bulk soil. In contrast, occluded fractions were only weakly or very weakly related to heavy and free light fractions and bulk soils.

3.4. Effect of treatment and time in bulk soils

The bulk soil dataset included $\Delta^{14}C$ values from four depths (0–15, 15–30 cm, 30–60 cm, and 60–90 cm) rather than the two uppermost depths that were density separated. A comparison of bulk $\Delta^{14}C$ values at the Walker Branch site (*control* and *litter* treatments) indicated no significant treatment effect ($p = 0.6076$) with the only significant fixed effect being depth ($p < 0.0001$). A comparison of bulk $\Delta^{14}C$ values at the Tennessee Valley site (*roots* and *roots + litter* treatments) indicated that $\Delta^{14}C$ values varied as a function of multiple parameters including treatment ($p = 0.0445$), depth ($p < 0.0001$), year ($p = 0.0335$), and year*depth ($p = 0.0547$). A summary of fixed effect statistics is given in supplementary Table 2.

3.5. Differences in incorporation of label in 1.7–2.4 g cm^{-3} and > 2.4 cm^{-3} fractions

A comparison of 1.7–2.4 and > 2.4 fractions across treatments and depths yielded several interesting differences (Table 5). The 1.7–2.4 fraction accounted for 4% (+/- 3% standard deviation) of the overall

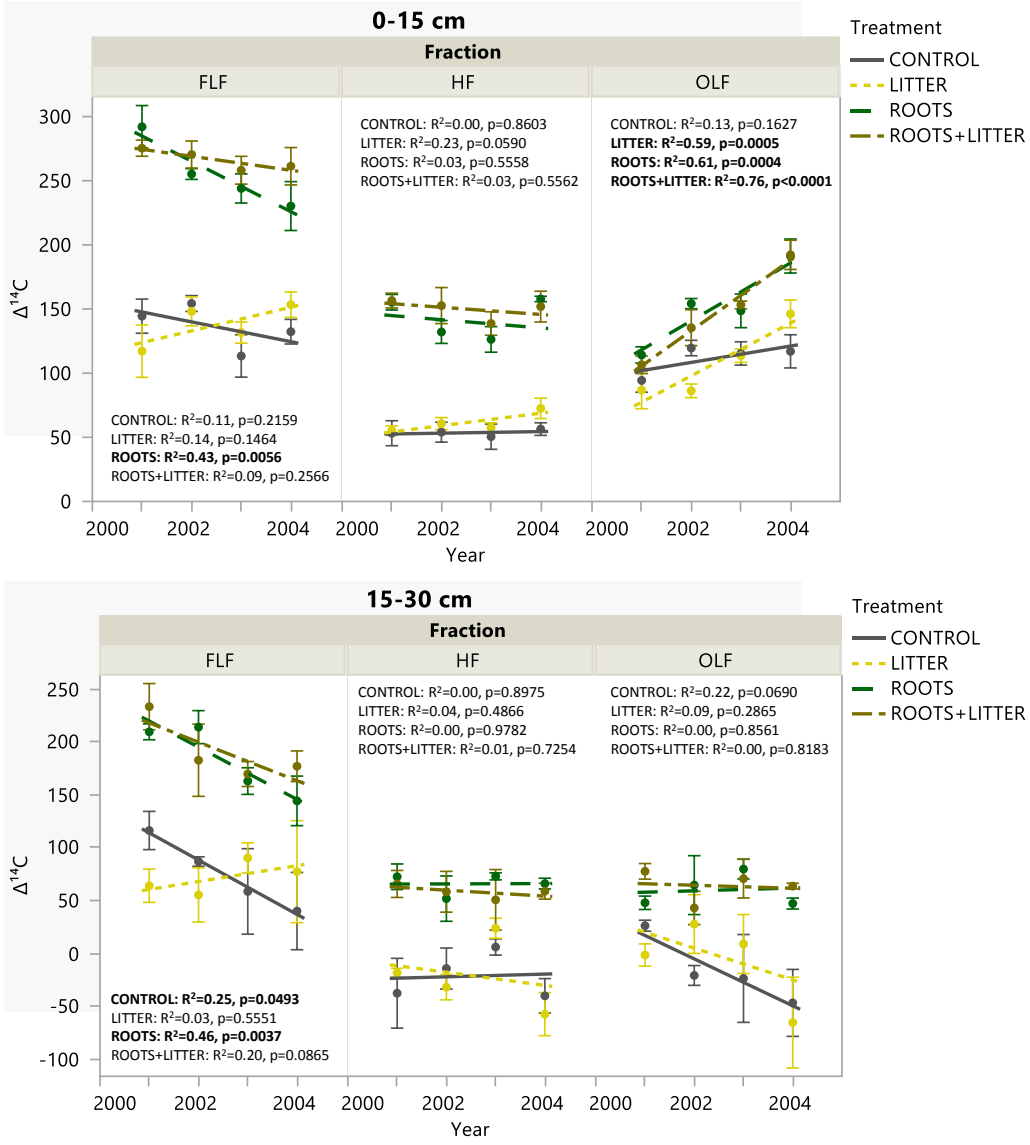

Fig. 5. Average and standard error $\Delta^{14}C$ (‰) values of fractions across time for two depth increments. Significant changes over time (bolded) at the 0–15 cm depth include a decline in the *roots* FLF over time, and increases in the OLF of the *litter, roots,* and *roots + litter* treatments. Note the increased variability at 22.5 cm of depth where the only significant changes over time were a decline in the *control*, and *roots* FLF over time.

heavy fraction mass. The > 2.4 fraction accounted for 96% (+/- 4% standard deviation) of the overall heavy fraction mass. C concentrations of 1.7–2.4 fractions were two orders of magnitude larger than C concentrations of the > 2.4 fraction (14.21% vs. 0.37%). Though the masses of the two fractions were very different, total C was more-or-less evenly distributed between the 1.7–2.4 and > 2.4 fractions, at 55% +/- 16% and 45% +/-16%, respectively (Table 5). N concentrations were 20 times higher in the 1.7–2.4 fractions as in the > 2.4 fractions (0.60% vs. 0.03%). Mean C/N molar ratios were significantly lower in the > 2.4 fraction (14 vs. 28), and $\delta^{13}C$ values were significantly higher (~-25‰ vs. ~ -27‰).

A statistical comparison of the 1.7–2.4 and > 2.4 fractions among the three treatments could not be made, due to differences in the initial plume strength at the two sites. A qualitative comparison, however, did suggest some trends. Values of $\Delta^{14}C$ appear to be elevated in the *roots* treatment in comparison to the *control* and *litter* treatments (Fig. 7, Supp. Table 3). The combined 1.7–2.4 and > 2.4 fractions from *Control* and *litter* treatments were compared using a simple *t* test and were found to not be different at the $\alpha = 0.05$ level ($p = 0.7436$; i.e no litter treatment effect). The $\Delta^{14}C$ values of the 1.7–2.4 and > 2.4 fractions show a nearly one-to-one positive relationship with each other. In the *roots* treatment, the uptake of label and the relative increase in $\Delta^{14}C$ was similar in both fractions.

3.6. Estimates of turnover times

Average steady-state estimates of turnover time based on natural abundance measurements for the 0–15 cm density fractions were 37, 71, and 108 years for free light, occluded and heavy fractions, respectively. Estimations based on the rate of incorporation of label in the *roots* treatment plots were substantially shorter. Data suggested that after a year and a half, up to 79% of free light C was derived from labeled material. Heavy and occluded fractions showed lower incorporations of 37% and 8%, respectively. Turnover times based on these rates of incorporation were 2, 4, and 18 years for free light, heavy and occluded fractions, respectively.

3.7. Compilation of current and prior measurements

A substantial number of studies have utilized the EBIS experimental

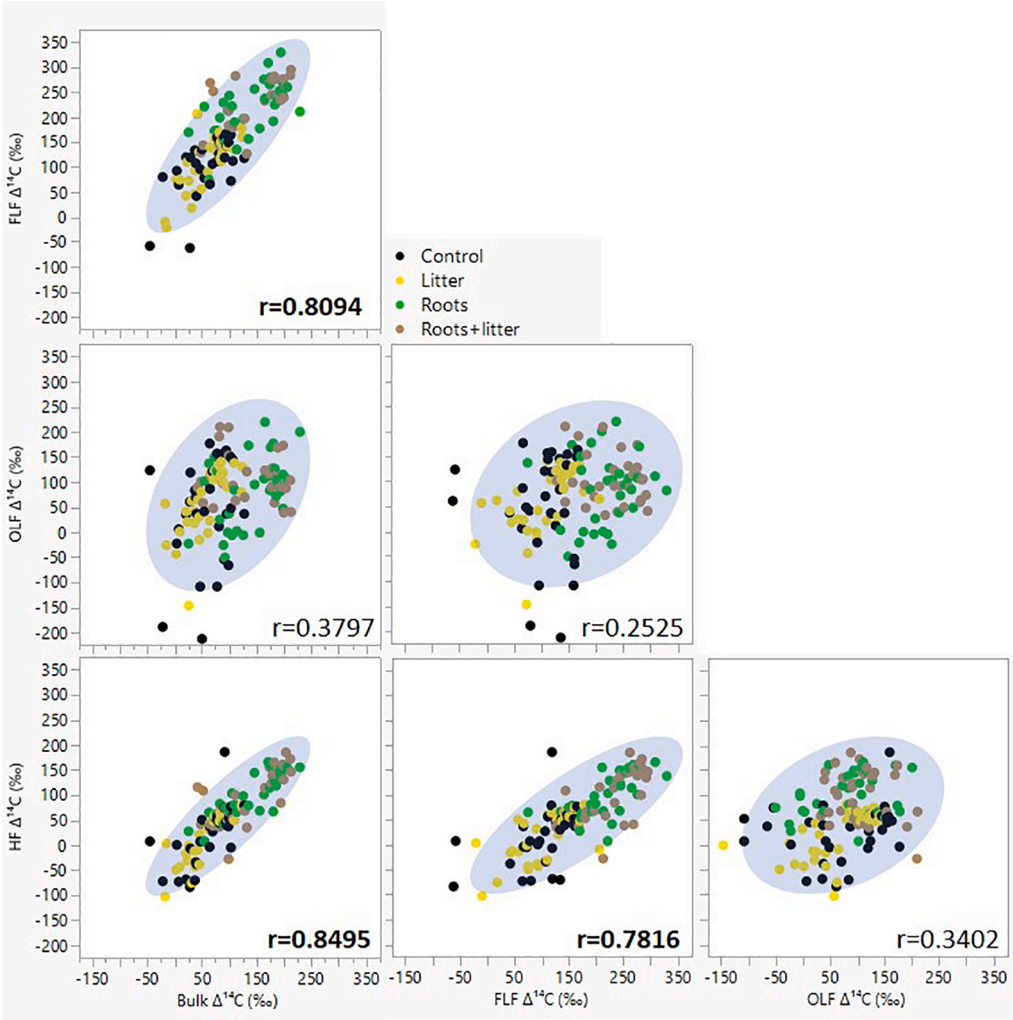

Fig. 6. Correlation plots exploring relationships among fraction and bulk soil $\Delta^{14}C$ values. Density ellipses encompassing 95% of the dataset are in light blue. Pearson correlation coefficients (r) are given within each panel. Bolded values are associated with a strong relationship between the variables.

Table 5
Characteristics of 1.7–2.4 and > 2.4 fractions ($n = 24$ for each fraction), errors are reported as standard deviation.

	Fraction				
	1.7–2.4 g cm^{-3}		>2.4 g cm^{-3}		
	Mean	±	Mean	±	*p*-value
Mass distribution (%)*	4	4	95	4	<0.0001
% C*	14.21	2.62	0.37	0.15	<0.0001
C distribution*	55	16	45	16	0.0325
% N*	0.6	0.08	0.03	0.01	<0.0001
N distribution*	40	20	60	20	0.0011
C/N (molar)*	28	5	14	2	<0.0001
$\delta^{13}C$*	−27.34	0.37	−25.39	0.63	<0.0001
$\delta^{15}N$	4.79	0.91	5.04	1.73	0.5316

* indicates significant difference determined by paired *t*-test, $\alpha = 0.05$.

site and/or materials taken from the site. These studies focused on different components of the ecosystem such as roots, microbial biomass, DOC, and soil respired CO_2 (references given in Supplementary Table 4). In order to examine and illustrate possible connections among different C cycle pools, we present compiled illustrations of C stocks, $\Delta^{14}C$, and hypothetical connections among the measured pools (Supplementary Figs 7–11).

4. Discussion

4.1. In this temperate forest ecosystem, what is the largest source of C to soil: Litter or roots?

This unique whole-system labelling experiment indicates that roots are the primary source of mineral soil C, while contributions of leaf litter to subsoil C pools are minimal. Though percolation of litter-derived dissolved phase C may contribute significantly to soil C stocks in some systems, such as northern hardwood forests (Rothstein et al., 2018), only a small uptake of tracer by dissolved organic C was detected in the *litter* treatment (Fröberg et al., 2007). Results from both bulk soils and soil fractions support the conclusion that roots are the source of the majority of C in mineral soils at these sites, as has been suggested by previous work in these and other soils (Kramer et al., 2010; Liebmann et al., 2020; Pausch and Kuzyakov, 2018). Though explicit statistical comparisons cannot be drawn between Walker Branch and Tennessee Valley because of variable exposures to the enrichment label, soil $\Delta^{14}C$ values at Tennessee Valley can logically be assumed to have been highly similar to values at the Walker Branch *control* treatment prior to the labelling event in 1999 (Trumbore et al., 2002), and a qualitative comparison indicates large differences in $\Delta^{14}C$ values of bulk soils and fractions between the two sites (Tables 3, 4, Figs. 4, 5). Consistent ratios of root biomass to bulk C stocks, ranging from ~ 4–7 across the four sampled depth increments (Supp. Fig. 7) suggest inputs are at steady state, but with total

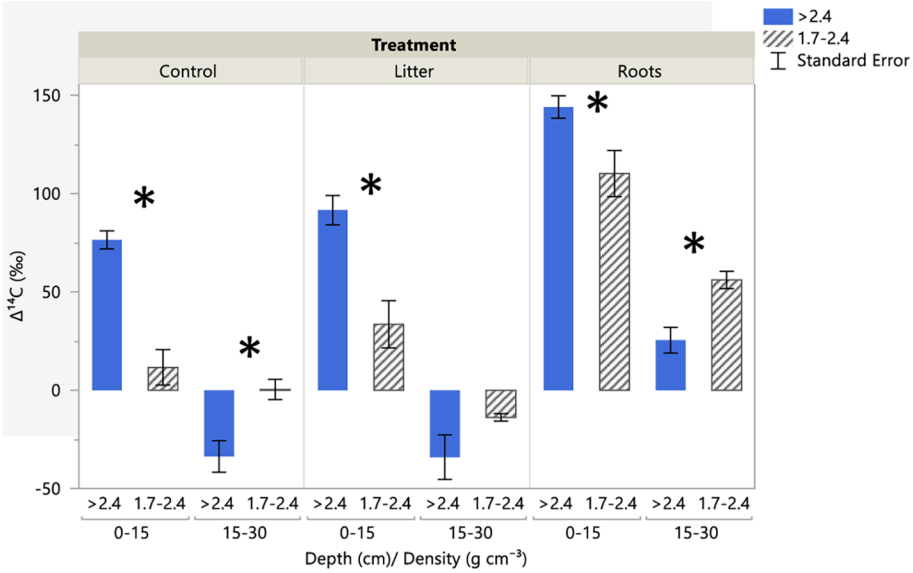

Fig. 7. Means and standard errors of $\Delta^{14}C$ values across treatments and depths. * indicates significant difference between fractions within an individual depth and treatment as determined by paired *t*-test, $\alpha = 0.05$.

inputs, input rates, and therefore bulk C stocks decreasing with depth. Radiocarbon abundance also decreases with depth, suggesting the rate of input must also be decreasing with depth. This is consistent with the idea that turnover of bulk SOC is directly related to the magnitude of fresh inputs (Fontaine et al., 2007).

Measures of microbial biomass and respired $^{14}CO_2$ from previous publications (Cisneros-Dozal et al., 2006; Hanson et al., 2006; Kramer et al., 2010) (Supplementary Fig. 12) additionally suggest that roots are not only the main source of stabilized SOC but are also the main source of microbial substrate (Kramer et al., 2010). Microbial metabolism drives SOC turnover while also contributing to a significant pool of subsoil organic matter in the form of microbial necromass (Cotrufo et al., 2013). However, tracking the flow of substrate to and through microbial biomass is nontrivial (Dannenmann et al., 2009; Kögel-Knabner, 2017). Here, differences in label uptake by microbes among treatments allows for identification of their main substrate sources. Based on $^{14}CO_2$ measurements of incubated surface soils (0–5 cm, A horizon), after 4 years of labeled litter additions, only ~ 6% of microbial biomass was derived from labeled Oi carbon (Kramer et al., 2010). Additionally, the $\Delta^{14}C$ values of fine roots reported by Joslin et al. (2006) were found to closely resemble the $\Delta^{14}C$ values of the PLFA compounds extracted from soils at each site, further suggesting that roots are the main substrate for the soil microbial community (Supplementary Figs. 10, 11).

Results suggest a small but significant contribution of DOC from Oi layers to the subsurface C cycle. Contributions of DOC from newly added litter to mineral soils are evident based on DOC measurements from previous work (Fröberg et al., 2009; Fröberg et al., 2007). However, these DOC contributions were not significant enough to alter the $\Delta\ ^{14}C$ values of the bulk soils. The lack of change in bulk soil $\Delta\ ^{14}C$ combined with measurement of microbial biomass and soil respired CO_2 $\Delta\ ^{14}C$ values (Cisneros-Dozal et al., 2006; Kramer et al., 2010) (Supplementary Figs. 8–11) suggested that DOC was being almost wholly consumed as a microbial growth substrate. This is consistent with work illustrating the dynamic nature of the water-soluble fraction of SOC (van der Voort et al., 2019).

Radiocarbon label derived from labeled litter additions (presumably in the form of labeled DOC) did not result in a treatment effect in the bulk soils of the *control* vs. *litter* treatments at Walker Branch but did result in a treatment effect in the bulk soils between *roots* and *roots* + *litter* treatments at Tennessee Valley (Supp. Table 2). This is most likely the result of differences in the timing of the implementation of the treatments at the two sites. Tennessee Valley was receiving an unknown amount of tracer 1–2 years prior to installation of treatments at Walker Branch, therefore labeled DOC may have had a substantially longer period of time to translocate to depth in the *roots* + *litter* treatment than in the *litter* treatment. This conclusion is supported by the significance of year*depth in the bulk soils model at Tennessee Valley, which was not significant at Walker Branch. Ultimately, though, this statistical significance highlights that while our experiment was able to detect an isotopic signal of DOC translocation, it only indicates that this process is occurring and does not address the quantity of C that was translocated vertically. Earthworms have been shown to affect label movement in similar studies, as some species harvest leaf litter and transport the material to depth (McFarlane et al., 2013; Wenk et al., 2016). In the current study, we are confident in assigning vertical transport of label to the movement of dissolved organics as opposed to bioturbation, as there is a clear distinction between litter layers of differing decomposition status, and a clear abrupt boundary at the organic/mineral soil interface.

4.2. Are there significant differences in annual C uptake among different pools/fractions of SOC? How quickly does newly introduced C propagate to depth? How deep does it go?

At the 0–15 cm depth increment, free light, occluded light and heavy fractions all varied in their incorporation of new annual C inputs at Tennessee Valley (*roots* and *roots* + *litter* treatments). Most notably, the free light and heavy fractions showed significant incorporation of label at the first sampling period in 2001, approximately 1.5 years following the labeling event. The occluded light fraction, however, incorporated label more slowly, with $\Delta^{14}C$ values increasing steadily over time (Fig. 5). The free light fraction of the *roots* treatment declined over time, but the free light fraction of the *roots* + *litter* treatment did not. In the litter treatment at Walker Branch, the heavy fraction never indicated incorporation of label, but the surface occluded light fractions do appear to increase slightly over time (Fig. 5). Label was detected at the 15–30 and 30–45 cm depth increments within 1.5 years as well, suggesting new inputs directly at depth, rather than transfer of inputs from more shallow layers to deeper layers.

Historical (Golchin et al., 1994; Tipping et al., 2012) models once postulated a passage of new inputs from the free light through the occluded and into the mineral-stabilized heavy fraction. The current results show that in A and E horizons (the 0–15 cm sampling layer), root-

derived C is introduced to both the free light and heavy fractions simultaneously, and SOC does not appear to pass through the occluded light fraction into the heavy fraction, as evidenced by an immediate incorporation of label into the heavy fraction and a delayed incorporation of label into the occluded light fraction which appeared to increase over time (Fig. 5). More recent conceptual models suggest a tightly-bound inner layer of organics directly associated with mineral surfaces, coated with increasing exchangeable layers of organics as distance from the mineral surface increases (i.e. the "onion model" (Kleber et al., 2007; Sollins et al., 2006)). This previous work illustrated a steady decline in radiocarbon abundance with increasing particle density across a variety of soils, suggesting that sequentially separating soils into denser and denser fractions allows for the partitioning of mineral-associated organics into fractions with increasingly thinner organic matter coatings and slower turnover as density increases (Sollins et al., 2009). Based on these observations, we expected the densest fraction (>2.4 g cm^{-3}) to have the thinnest, most tightly-bound organic layer which would also be the most depleted in radiocarbon, but this was not the case. Not only was the > 2.4 fraction enriched in radiocarbon in comparison to the 1.7–2.4 fraction in the 0–15 cm depth, it also incorporated more label than the 1.7–2.4 fraction in the *roots + litter* treatment. This substantial uptake of label in the densest portion of the mineral-associated fraction in this study (>2.4 g cm^{-3}) suggests that all organics from all densities and degrees of mineral association have a significant portion of C which cycles on a near-annual basis. This is additional empirical evidence of the heterogeneous nature of SOC fractions and suggests that regardless of how finely (sequentially density separated) a soil is partitioned, fractions may always contain a substantial portion of C with fast turnover.

Given that roots are the source of the majority of SOC, and that changes in the $\Delta^{14}C$ values of fractions are indicative of an incorporation of root-derived labeled C, this simple rate of transfer can be used to estimate turnover of C in each fraction. This approach yielded mean residence times of 2, 4, and 18 years for free light, heavy and occluded fraction from the 0–15 cm depth. Additionally, incorporation rates associated with the uptake of tracer indicate that substantial amounts of new organics are incorporated into fractions on a yearly basis (79% of total C in the free light, 8% in the heavy, and 37% in the occluded).These timespans are very short in comparison to steady state mean residence times calculated from natural abundance $\Delta^{14}C$ values taken from unlabeled soil near the two sites (Johnson et al., 2008) (0–15 cm), and using a steady-state mean residence time model (Torn et al., 2002; Trumbore, 1993), which averaged 37, 108, and 71 years for free light, heavy, and occluded fractions. Both calculations indicate differences in the annual incorporation of new C by the individual fractions, however the two estimations agree neither in magnitude nor pattern. This discrepancy among methods for estimating SOC turnover is well documented in (Feng et al., 2016), which used a *meta*-analysis of 52 studies to examine how mean residence time estimations vary according to methodology. Their analysis indicated that estimates based on natural abundance radiocarbon values were consistently 2–4 times greater than estimates based on C3/C4 vegetation switches and up to an order of magnitude greater than estimates derived from soil incubations. We speculate that the discrepancies in our own dataset result from non-homogeneity within each of the density fractions, with a portion of each experiencing very rapid turnover and other portions having much longer mean residence times. This proposition is strongly supported by the consistent and significant differences in composition and radiocarbon abundance between the 1.7–2.4 g cm^{-3} and > 2.4 g cm^{-3} fractions (Table 5, Fig. 7).

The influence of time on the transfer of label to depth was not immediately interpretable. Treatment, and year*depth were both significant effects in the Tennessee Valley Authority bulk soil model (Supp table 2), with the *roots + litter* treatment incorporating more label relative to the *roots* treatment. However, changes in $\Delta^{14}C$ values over time fluctuated instead of increasing steadily over time (Fig. 3) Label was detected at up to 45 cm of depth in the Tennessee Valley plots at the time of the first sampling, though was never detected below this depth even after 4 years of label incorporation, suggesting a very slow rate of incorporation of new photosynthates in deep soils during this experiment. The rate of label incorporation decreased with increasing depth in general, which is consistent with decreases in annual root inputs with increasing depth (Joslin et al., 2006).

4.3. How are different pools/fractions related to one another? How is C transferred among them? Do the two mineral-associated SOC pools show similar tracer incorporation?

Previous work utilizing natural abundance radiocarbon measurements indicate that free light fractions cycle much more rapidly than heavy fractions. However, this whole system labelling experiment indicates that free light and heavy fractions share the same pathways of incorporation of newly fixed photosynthates, namely direct annual input from roots, whether as particulate litter (FLF) or molecular-scale residues and exudates (HF). This is further illustrated by strong correlations among free light and heavy fraction $\Delta^{14}C$ values (Fig. 6). These relationships are absent in the occluded light $\Delta^{14}C$ values which show only very weak relationships with other fractions or bulk $\Delta^{14}C$ values. Therefore, our data suggests occluded light fraction incorporates C from the free light and heavy fractions over a multi-year time span, which we hypothesize occurs through the process of aggregate turnover. This is consistent with more contemporary modeling efforts where C is incorporated into aggregates from both particulate and mineral-associated pools (Abramoff et al., 2018). We observed a strong relationship between heavy fraction and bulk soil $\Delta^{14}C$ values, emphasizing the strong dependence of bulk C turnover rates on the mineral-associated pool which often comprises the majority of C in mineral soils, e.g.(Swanston et al., 2005).

Based on previous work with sequential density separations (see "onion model" above), we expected the further partitioning of the heavy fraction into the sub-fractions of differing density (1.7–2.4 g cm^{-3} and > 2.4 g cm^{-3}) to result in isolation of an "inert" densest fraction (>2.4 g cm^{-3}). Characteristics of the > 2.4 cm^{-3} fraction suggest a higher degree of microbial processing than the 1.7–2.4 cm^{-3} fraction (i.e. narrowed C/N in combination with higher $\delta^{13}C$ values, Table 5), consistent with previous observations (Kleber et al., 2011; Sollins et al., 2009; Sollins et al., 2006). This pattern of increasing degree of microbial processing with increasing heavy fraction density would seem to suggest differences in rates and pathways of incorporation of C into these sub-fractions. However, both of these fractions indicate incorporation of label in the *roots* treatment, and that this incorporation is of similar magnitude. Similar rates of incorporation of label in combination with differing degrees of microbial processing would seem to be contradictory. Again, we interpret this discrepancy as evidence of non-homogeneity within each of these sub-fractions, with each sub-fraction containing portions of faster- and more slowly-cycling organics.

Of note is the reversal in the relative $\Delta^{14}C$ abundances of the two sub-fractions between the 0–15 cm and 15–30 cm depths (Fig. 7). We hypothesize that this may be the result of the propagation of bomb pulse-derived radiocarbon through the soil. This "bomb" C has been shown to act as a reactive tracer, moving from more rapidly-cycling pools to more slowly cycling pools over a period of years to decades, e.g. (Trumbore and Zheng, 1996). Results suggest that this bomb pulse is being propagated to the 1.7–2.4 and > 2.4 fractions at different rates.

4.4. How do these results fit into an ecosystem context?

This experiment was conducted at a site that is arguably one of the best-studied forest landscapes in the literature. Studies most relevant to the current study address C and nutrient stocks in experimental harvests (e.g. Johnson et al., 1988; Johnson and Todd, 1998), precipitation manipulations (e.g., Johnson et al. 2008; Fröberg et al., 2007) and untreated control forests that span a wide range of parent materials,

geomorphic settings, and soil and vegetation types (Trettin et al., 1999). Our study was conducted in typical residual Ultisols formed in the limestone and dolomite saprolite of upper slope and ridge positions, under the most widely distributed, oak-dominated vegetation at ORR (Trettin et al., 1999). This suggests that the soil C dynamics we report here are representative of much of the local landscape, and transferable to sites with similar physiography (well-drained, weathered uplands) across the wider Appalachian Ridge and Valley province.

Regarding deep soil processes, current results shed light on nutrient dynamics observed at this site. Steady levels of Ca in vegetation, in comparison to depleted Ca levels in these weathered soils, implied the presence of deep roots able to mine C at depth. However, deep roots are rarely encountered during sampling (Johnson et al., 1988; Johnson and Todd 1998; Johnson et al. 2008; Trettin et al., 1999). Bulk soils sampled from 60 to 90 cm indicated no incorporation of tracer over the course of our experiment (Fig. 4), but root ($\Delta^{14}C$ = 339 ‰; stock = 85 g_C m^{-2}, Joslin et al., 2006) and DOC pools ($\Delta^{14}C$ = 114–257 ‰; stock = 2 g_C m^{-2}, Fröberg et al., 2007) at this depth interval were strongly enriched, suggesting that roots are active and persistent at depth, consistent with the idea of "hotspots" of preferential rooting.

Regarding temporal dynamics, many ORR researchers have reported surprising inter-annual to decadal fluctuations in C and nutrient pools that are usually assumed to be at steady state (Johnson et al. 2007). Researchers have not been able to fully explain these observed temporal fluctuations, or how these dynamics may influence the rates, pathways (e.g., litter vs. root), and forms (e.g., soil C fractions) of C accumulation and turnover in soil over longer timescales than our study. The comparison of C turnover times using our tracer approach vs. natural abundance $\Delta^{14}C$, suggests processes and drivers operating over different timescales which may explain the previously observed variation.

Overall, the location of our experiment on a widely distributed soil type maximizes its relevance to temperate forest ecosystems more broadly and can inform future radiocarbon-derived assessments of SOC inputs and turnover. As such, this opportunistic case study took advantage of an unexpected event to gain new insights into C cycling in a common ecosystem type.

5. Summary

Radiocarbon measurements of mineral soils and mineral soil density fractions following a whole system labeling experiment support evidence that roots are the primary source of SOC in these weathered soils of oak-dominated temperate forests. Significant amounts of newly fixed photosynthates are incorporated into free light and heavy fractions on an annual basis in these soils. Results also suggest that C does not seem to pass through the occluded fraction into the heavy fraction. Rather C passes through the free light and heavy fractions into the occluded fraction, likely through the process of aggregate turnover. Additionally, all densities of the mineral-associated fraction incorporate new inputs on an annual basis. Because SOC is primarily derived from roots in these soils, rates of incorporation of new C decrease with depth concomitant with decreases in live and dead rootstocks.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2021.115385.

References

Abramoff, R., Xu, X., Hartman, M., O'Brien, S., Feng, W., Davidson, E., Finzi, A., Moorhead, D., Schimel, J., Torn, M., Mayes, M.A., 2018. The Millennial model: in search of measurable pools and transformations for modeling soil carbon in the new century. Biogeochemistry 137 (1-2), 51–71.

Bird, J.A., Kleber, M., Torn, M.S., 2008. 13C and 15N stabilization dynamics in soil organic matter fractions during needle and fine root decomposition. Org Geochem. 39 (4), 465–477.

Bradford, M.A., Wieder, W.R., Bonan, G.B., Fierer, N., Raymond, P.A., Crowther, T.W., 2016. Managing uncertainty in soil carbon feedbacks to climate change. Nat. Clim. Change 6 (8), 751–758.

Cary, N., 2011. SAS Institute Inc SAS/STAT® 9.3 user's guide: the MIXED procedure (Chapter). SAS Institute Inc.

Cisneros-Dozal, L.M., Trumbore, S., Hanson, P.J., 2006. Partitioning sources of soil-respired CO2 and their seasonal variation using a unique radiocarbon tracer. Glob. Change Biol. 12 (2), 194–204.

Cook, G.T., Scott, E.M., Harkness, D.D., 2009. Radiocarbon as a tracer in the global carbon cycle. Radioactivity in the Environment 16, 89–137.

Cotrufo, M.F., Wallenstein, M.D., Boot, C.M., Denef, K., Paul, E., 2013. The M icrobial E fficiency-M atrix S tabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: do labile plant inputs form stable soil organic matter? Glob. Change Biol. 19 (4), 988–995.

Crow, S.E., Swanston, C.W., Lajtha, K., Brooks, J.R., Keirstead, H., 2007. Density fractionation of forest soils: methodological questions and interpretation of incubation results and turnover time in an ecosystem context. Biogeochemistry 85 (1), 69–90.

Curtis, P.S., Hanson, P.J., Bolstad, P., Barford, C., Randolph, J.C., Schmid, H.P., Wilson, K.B., 2002. Biometric and eddy-covariance based estimates of annual carbon storage in five eastern North American deciduous forests. Agric. For. Meteorol. 113 (1-4), 3–19.

Dannenmann, M., Simon, J., Gasche, R., Holst, J., Naumann, P.S., Kögel-Knabner, I., Knicker, H., Mayer, H., Schloter, M., Pena, R., Polle, A., Rennenberg, H., Papen, H., 2009. Tree girdling provides insight on the role of labile carbon in nitrogen partitioning between soil microorganisms and adult European beech. Soil Biol. Biochem. 41 (8), 1622–1631.

Davidson, E.A., Savage, K., Bolstad, P., Clark, D.A., Curtis, P.S., Ellsworth, D.S., Hanson, P.J., Law, B.E., Luo, Y., Pregitzer, K.S., Randolph, J.C., Zak, D., 2002. Belowground carbon allocation in forests estimated from litterfall and IRGA-based soil respiration measurements. Agric. For. Meteorol. 113 (1-4), 39–51.

Davis, J.C., Proctor, I.D., Southon, J.R., Caffee, M.W., Heikkinen, D.W., Roberts, M.L., Moore, T.L., Turteltaub, K.W., Nelson, D.E., Loyd, D.H., Vogel, J.S., 1990. LLNL/UC AMS facility and research program. Nucl. Instrum. Methods Phys. Res., Sect. B 52 (3-4), 269–272.

Feng, W., Shi, Z., Jiang, J., Xia, J., Liang, J., Zhou, J., Luo, Y., 2016. Methodological uncertainty in estimating carbon turnover times of soil fractions. Soil Biol. Biochem. 100, 118–124.

Fontaine, S., Barot, S., Barré, P., Bdioui, N., Mary, B., Rumpel, C., 2007. Stability of organic carbon in deep soil layers controlled by fresh carbon supply. Nature 450 (7167), 277–280.

Fröberg, M., Hanson, P.J., Trumbore, S.E., Swanston, C.W., Todd, D.E., 2009. Flux of carbon from 14C-enriched leaf litter throughout a forest soil mesocosm. Geoderma 149 (3-4), 181–188.

Fröberg, M., Jardine, P.M., Hanson, P.J., Swanston, C.W., Todd, D.E., Tarver, J.R., Garten, C.T., 2007. Low dissolved organic carbon input from fresh litter to deep mineral soils. Soil Sci. Soc. Am. J. 71 (2), 347–354.

Golchin, A., Oades, J., Skjemstad, J., Clarke, P., 1994. Study of free and occluded particulate organic matter in soils by solid state 13C CP/MAS NMR spectroscopy and scanning electron microscopy. Soil Res. 32 (2), 285–309.

Griscom, B.W., Adams, J., Ellis, P.W., Houghton, R.A., Lomax, G., Miteva, D.A., Schlesinger, W.H., Shoch, D., Siikamäki, J.V., Smith, P., Woodbury, P., Zganjar, C., Blackman, A., Campari, J., Conant, R.T., Delgado, C., Elias, P., Gopalakrishna, T., Hamsik, M.R., Herrero, M., Kiesecker, J., Landis, E., Laestadius, L., Leavitt, S.M., Minnemeyer, S., Polasky, S., Potapov, P., Putz, F.E., Sanderman, J., Silvius, M., Wollenberg, E., Fargione, J., 2017. Natural climate solutions. Proc. Natl. Acad. Sci. 114 (44), 11645–11650.

Hanson, P.J., Huston, M.A., Todd, D.E., 2003. Walker branch throughfall displacement experiment, North American Temperate Deciduous Forest Responses to Changing Precipitation Regimes. Springer, pp. 8–31.

Hanson, P., Trumbore, S., Swanston, C., Torn, M.S., Jastrow, J., Parton, W., Post, W.M., Froberg, M.J., Hainsworth, L.J., Kleber, M., Kramer, C., Matamala-Paraceda, R.,

Garten, C.T., 2006. Summary of the 2006 Enriched Background Isotope Study (EBIS) Workshop, Argonne, Illinois. The FY2005 progress report to DOE's Office of Science, Biological and Environmental Research (BER), Terrestrial Carbon Processes (TCP), Oak Ridge National Laboratory.
Hanson, P.J., Wullschleger, S.D., Bohlman, S.A., Todd, D.E., 1993. Seasonal and topographic patterns of forest floor CO2 efflux from an upland oak forest. Tree Physiol. 13 (1), 1–15.
Harkness, D.D., Harrison, A.F., Bacon, P.J., 1986. The temporal distribution of 'bomb'14 C in a forest soil. Radiocarbon 28 (2A), 328–337.
Harkness, D.D., 1970. Artificial carbon-14, a tracer for carbon in the atmosphere and biosphere, ProQuest Dissertations & Theses.
Jastrow, J.D., Miller, R.M., Boutton, T.W., 1996. Carbon dynamics of aggregate-associated organic matter estimated by carbon-13 natural abundance. Soil Sci. Soc. Am. J. 60 (3), 801–807.
Johnson, D.W., Todd Jr, D.E., Hanson, P.J., 2008. Effects of throughfall manipulation on soil nutrient status: results of 12 years of sustained wet and dry treatments. Glob. Change Biol. 14 (7), 1661–1675.
Johnson, D., Todd Jr, D., 1998. Harvesting effects on long-term changes in nutrient pools of mixed oak forest. Soil Sci. Soc. Am. J. 62 (6), 1725–1735.
Johnson, D.W., Van Hook, R.I., 2012. Analysis of biogeochemical cycling processes in Walker Branch Watershed. Springer Science & Business Media.
Johnson, D.W., Henderson, G.S., Todd, D.E., 1988. Changes in nutrient distribution in forests and soils of Walker Branch watershed, Tennessee, over an eleven-year period. Biogeochemistry 5 (3), 275–293.
Joslin, J.D., Gaudinski, J.B., Torn, M.S., Riley, W.J., Hanson, P.J., 2006. Fine-root turnover patterns and their relationship to root diameter and soil depth in a 14C-labeled hardwood forest. New Phytol. 172 (3), 523–535.
Kleber, M., Nico, P.S., Plante, A., Filley, T., Kramer, M., Swanston, C., Sollins, P., 2011. Old and stable soil organic matter is not necessarily chemically recalcitrant: implications for modeling concepts and temperature sensitivity. Glob. Change Biol. 17 (2), 1097–1107.
Kleber, M., Sollins, P., Sutton, R., 2007. A conceptual model of organo-mineral interactions in soils: self-assembly of organic molecular fragments into zonal structures on mineral surfaces. Biogeochemistry 85 (1), 9–24.
Kögel-Knabner, I., 2017. The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter: fourteen years on. Soil Biol. Biochem. 105, A3–A8.
Kramer, C., Trumbore, S., Fröberg, M., Dozal, L.M.C., Zhang, D., Xu, X., Santos, G.M., Hanson, P.J., 2010. Recent (< 4 year old) leaf litter is not a major source of microbial carbon in a temperate forest mineral soil. Soil Biol. Biochem. 42 (7), 1028–1037.
Liang, C., Amelung, W., Lehmann, J., Kästner, M., 2019. Quantitative assessment of microbial necromass contribution to soil organic matter. Glob. Change Biol. 25 (11), 3578–3590.
Liebmann, P., Wordell-Dietrich, P., Kalbitz, K., Mikutta, R., Kalks, F., Don, A., Woche, S. K., Dsilva, L.R., Guggenberger, G., 2020. Relevance of aboveground litter for soil organic matter formation–a soil profile perspective. Biogeosciences 17 (12), 3099–3113.
McFarlane, K.J., Torn, M.S., Hanson, P.J., Porras, R.C., Swanston, C.W., Callaham, M.A., Guilderson, T.P., 2013. Comparison of soil organic matter dynamics at five temperate deciduous forests with physical fractionation and radiocarbon measurements. Biogeochemistry 112 (1-3), 457–476.
Minasny, B., Malone, B.P., McBratney, A.B., Angers, D.A., Arrouays, D., Chambers, A., Chaplot, V., Chen, Z.-S., Cheng, K., Das, B.S., Field, D.J., Gimona, A., Hedley, C.B., Hong, S.Y., Mandal, B., Marchant, B.P., Martin, M., McConkey, B.G., Mulder, V.L., O'Rourke, S., Richer-de-Forges, A.C., Odeh, I., Padarian, J., Paustian, K., Pan, G., Poggio, L., Savin, I., Stolbovoy, V., Stockmann, U., Sulaeman, Y., Tsui, C.-C., Vågen, T.-G., van Wesemael, B., Winowiecki, L., 2017. Soil carbon 4 per mille. Geoderma 292, 59–86.
O'brien, B.J., Stout, J.D., 1978. Movement and turnover of soil organic matter as indicated by carbon isotope measurements. Soil Biol. Biochem. 10 (4), 309–317.
Pausch, J., Kuzyakov, Y., 2018. Carbon input by roots into the soil: quantification of rhizodeposition from root to ecosystem scale. Glob. Change Biol. 24 (1), 1–12.
Paustian, K., Lehmann, J., Ogle, S., Reay, D., Robertson, G.P., Smith, P., 2016. Climate-smart soils. Nature 532 (7597), 49–57.
Peters, L., Grigal, D., Curlin, J., Selvidge, W., 1970. Walker branch watershed project: chemical, physical and morphological properties of the soils of walker branch watershed. Oak Ridge National Lab, Tenn.
Rasse, D.P., Rumpel, C., Dignac, M.-F., 2005. Is soil carbon mostly root carbon? Mechanisms for a specific stabilisation. Plant Soil 269 (1-2), 341–356.
Rothstein, D.E., Toosi, E.R., Schaetzl, R.J., Grandy, A.S., 2018. Translocation of Carbon from Surface Organic Horizons to the Subsoil in Coarse-Textured Spodosols: Implications for Deep Soil C Dynamics. Soil Sci. Soc. Am. J. 82 (4), 969–982.
Schmidt, M.W., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I.A., Kleber, M., Kögel-Knabner, I., Lehmann, J., Manning, D.A., 2011. Persistence of soil organic matter as an ecosystem property. Nature 478 (7367), 49–56.
Sierra, C.A., Müller, M., Trumbore, S.E., 2014. Modeling radiocarbon dynamics in soils: SoilR version 1.1. Geosci. Model Dev. 7 (5), 1919–1931.
Sollins, P., Kramer, M.G., Swanston, C., Lajtha, K., Filley, T., Aufdenkampe, A.K., Wagai, R., Bowden, R.D., 2009. Sequential density fractionation across soils of contrasting mineralogy: evidence for both microbial- and mineral-controlled soil organic matter stabilization. Biogeochemistry 96 (1-3), 209–231.
Sollins, P., Swanston, C., Kleber, M., Filley, T., Kramer, M., Crow, S., Caldwell, B.A., Lajtha, K., Bowden, R., 2006. Organic C and N stabilization in a forest soil: Evidence from sequential density fractionation. Soil Biol. Biochem. 38 (11), 3313–3324.
Spielvogel, S., Prietzel, J., Kögel-Knabner, I., 2016. Stand scale variability of topsoil organic matter composition in a high-elevation Norway spruce forest ecosystem. Geoderma 267, 112–122.
Stuiver, M., Polach, H.A., 1977. Discussion reporting of 14 C data. Radiocarbon 19 (3), 355–363.
Swanston, C.W., Torn, M.S., Hanson, P.J., Southon, J.R., Garten, C.T., Hanlon, E.M., Ganio, L., 2005. Initial characterization of processes of soil carbon stabilization using forest stand-level radiocarbon enrichment. Geoderma 128 (1-2), 52–62.
Tipping, E., Chamberlain, P.M., Fröberg, M., Hanson, P.J., Jardine, P.M., 2012. Simulation of carbon cycling, including dissolved organic carbon transport, in forest soil locally enriched with 14 C. Biogeochemistry 108 (1-3), 91–107.
Torn, M.S., Lapenis, A.G., Timofeev, A., Fischer, M.L., Babikov, B.V., Harden, J.W., 2002. Organic carbon and carbon isotopes in modern and 100-year-old-soil archives of the Russian steppe. Glob. Change Biol. 8 (10), 941–953.
Treseder, K., Torn, M., Masiello, C., 2006. An ecosystem-scale radiocarbon tracer to test use of litter carbon by ectomycorrhizal fungi. Soil Biol. Biochem. 38 (5), 1077–1082.
Trettin Jr, C., Johnson, D., Todd, D., 1999. Forest Nutrient and Carbon Pools at Walker Branch Watershed Changes during a 21-Year Period. Soil Sci. Soc. Am. J. 63 (5), 1436–1448.
Trumbore, S., Gaudinski, J.B., Hanson, P.J., Southon, J.R., 2002. Quantifying ecosystem-atmosphere carbon exchange with a 14C label. Eos, Transactions American Geophysical Union 83 (24), 265–268.
Trumbore, S.E., 1993. Comparison of carbon dynamics in tropical and temperate soils using radiocarbon measurements. Global Biogeochem. Cycles 7 (2), 275–290.
Trumbore, S.E., Zheng, S., 1996. Comparison of fractionation methods for soil organic matter 14 C analysis. Radiocarbon 38 (2), 219–229.
van der Voort, T.S., Mannu, U., Hagedorn, F., McIntyre, C., Walthert, L., Schleppi, P., Haghipour, N., Eglinton, T.I., 2019. Dynamics of deep soil carbon–insights from 14C time series across a climatic gradient. Biogeosciences 16 (16), 3233–3246.
Vogel, J.S., Southon, J.R., Nelson, D.E., 1987. Catalyst and binder effects in the use of filamentous graphite for AMS. Nucl. Instrum. Methods Phys. Res., Sect. B 29 (1-2), 50–56.
von Lützow, M., Kögel-Knabner, I., Ekschmitt, K., Flessa, H., Guggenberger, G., Matzner, E., Marschner, B., 2007. SOM fractionation methods: relevance to functional pools and to stabilization mechanisms. Soil Biol. Biochem. 39 (9), 2183–2207.
Wagai, R., Mayer, L.M., Kitayama, K., 2009. Nature of the "occluded" low-density fraction in soil organic matter studies: A critical review. Soil Science and Plant Nutrition 55 (1), 13–25.
Wenk, E.S., Callaham, M.A., O'Brien, J.J., Hanson, P.J., 2016. Soil macroinvertebrate communities across a productivity gradient in deciduous forests of eastern North America. Northeastern Naturalist 23 (1), 25–44.