

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

**Authors:** Katherine A. Heckman<sup>1,2\*</sup>, Christopher W. Swanston<sup>1,2</sup>, Margaret S. Torn<sup>3</sup>, Paul J. Hanson<sup>4</sup>, Lucas E. Nave<sup>5,2</sup>, Rachel C. Porras<sup>3</sup>, Umakant Mishra<sup>6</sup>, Markus Bill<sup>3</sup>

**Affiliations:**

<sup>1</sup>USDA Forest Service, Northern Research Station, Houghton, MI 49931

<sup>2</sup>Northern Institute of Applied Climate Science, USDA Forest Service, Houghton, MI 49931

<sup>3</sup>Lawrence Berkeley National Laboratory, Earth & Environmental Sciences Area, Berkeley, CA 94720

<sup>4</sup>Oak Ridge National Laboratory, Climate Change Science Institute, Oak Ridge, TN 37831

<sup>5</sup>University of Michigan, Biological Station and Department of Ecology and Evolutionary Biology, Pellston, MI 49769

<sup>6</sup>Computational Biology & Biophysics, Sandia National Laboratories, Livermore, CA, 94550

\*Corresponding author

**Keywords:** soil organic matter, radiocarbon, Enriched Background Isotope Study, density fractionation, C stabilization

**For submission to:** *Geoderma*

1       **ABSTRACT:**

2           To a large degree, the sources and stability of soil organic carbon remain poorly  
3       constrained. A clear understanding of links among the components of the soil C cycle is  
4       hampered by the complexity of the system as well as challenges associated with partitioning  
5       bulk soil C into meaningful fractions. A large accidental <sup>14</sup>CO<sub>2</sub> release at the Oak Ridge  
6       Reservation in Tennessee, USA provided a strong label pulse into adjacent, well-studied oak  
7       forests, resulting in highly elevated  $\Delta^{14}\text{C}$  values in leaf litter (~1000‰) and roots (~260-450‰).  
8       A four-year manipulative study was conducted to determine the relative contribution of litter  
9       versus roots to the bulk mineral soil C pool, as well as to free light, occluded light and heavy  
10       fractions. The heavy fraction was further split into fractions with densities of 1.7-2.4 g cm<sup>-3</sup> and  
11       >2.4 g cm<sup>-3</sup> to test the homogeneity of the mineral-associated fraction of C. Substantial  
12       concentrations of label were detected in all soil fractions within a year of the <sup>14</sup>CO<sub>2</sub> release,  
13       indicating rapid incorporation of newly fixed photosynthates in all fractions of soil organic C,

14 regardless of differences in stability inferred by previous work. This rapid incorporation of label  
15 occurred only in treatments where roots were labeled, indicating that roots are the major source  
16 of inputs to mineral soil C stocks at these sites. Separation of the heavy fraction into  
17 subfractions of intermediate (1.7-2.4 g cm<sup>-3</sup>) and high (>2.4 g cm<sup>-3</sup>) density indicated that both  
18 subfractions incorporated label at similar rates, despite significant differences in degree of  
19 microbial processing. In general, the rate of label incorporation suggested a much faster  
20 turnover for all fractions than indicated by natural radiocarbon abundance values. This suggests  
21 that within each soil fraction there are portions of slow-cycling and fast-cycling materials, and  
22 the determination of an average turnover time or mean age is dependent on experimental  
23 approach. The rapid incorporation of label into all fractions within a year regardless of inferred  
24 stability implies a high degree of heterogeneity in all fractions regardless of how finely the soils  
25 are partitioned. Further refinement of the nature and drivers of this heterogeneity could yield  
26 important insights into the soil C cycle.

27

## 28 **INTRODUCTION:**

29 Maintaining, and if possible, increasing soil organic C (SOC) stocks has garnered  
30 increasing interest as a potential climate change mitigation strategy (Griscom et al., 2017;  
31 Minasny et al., 2017; Paustian et al., 2016). However, our ability to accurately model and  
32 manage SOC stocks is hindered by a lack of clarity on some of the most basic processes  
33 regulating SOC stocks. For example, previous research has suggested that the relative  
34 importance of different C sources to the soil (e.g. roots versus litter) varies with ecosystem  
35 factors such as vegetation composition and soil properties, but the magnitude (and even the  
36 very direction) of these differences is not currently well defined (Bradford et al., 2016).

37 Measurements across several adjacent forest vegetation types on loamy, high-activity Ultisols  
38 indicated litter inputs were consistently restricted to topsoils; root inputs varied with depth and  
39 dominant tree species and were the dominant source of SOM in subsoils (Spielvogel et al.,

40 2016). In contrast, examination of coarse-textured Spodosols under two forest vegetation types  
41 indicated that dissolved organic C from litter accounted for 80-95% of total C inputs to subsoils,  
42 varying with dominant tree species (Rothstein et al., 2018). These differences in C source have  
43 implications for the quality and stability of resulting SOC stocks. Specifically, a meta-analysis of  
44 forest and grassland litterbag and incubation studies suggested that root-derived SOC persists  
45 in soils an average of 2.4 times longer than shoot-derived SOC (Rasse et al., 2005). Similarly, a  
46 subsequent study found 28% higher retention of introduced root material in comparison to  
47 needleleaf material in an incubation tracer experiment (Bird et al., 2008). The strong influence of  
48 C source on SOC persistence, in combination with the high variability of root versus litter inputs  
49 across vegetation and soil types suggests further investigation across a diversity of soils is  
50 needed.

51         Additionally, though many conceptual models of the SOC cycle have been proposed, the  
52 actual pathways of C movement and transfer among pools of differing stability have not been  
53 definitively established due to the large number of confounding and/or interacting processes and  
54 controls at work in any individual soil (Schmidt et al., 2011). Some models separate bulk SOC  
55 into subfractions or pools of differing stability, which may include fractions representing  
56 unprotected particulate organic matter (free/light fraction), particulate organic matter occluded  
57 within aggregates (occluded light fraction), and organic matter associated with mineral surfaces  
58 (heavy fraction) (e.g.(Sierra et al., 2014)). These fractions correspond to those which can be  
59 feasibly physically separated from one another in the lab using a combination of density  
60 fractionation and aggregate disruption techniques (Golchin et al., 1994; Swanston et al., 2005),  
61 and which align conceptually with known mechanisms of soil C stabilization (e.g. (von Lützow et  
62 al., 2007). Historically, models suggested that new C inputs to soils first enter the free/light  
63 fraction then move through the occluded light fraction and are subsequently stabilized in the  
64 heavy fraction, e.g. (Golchin et al., 1994; Wagai et al., 2009). Though these pathways are

65 generally supported by observational studies, how they may vary across ecosystems and soils  
66 is not known.

67 One approach to elucidating the soil C cycle is through the addition of a reactive tracer,  
68 which allows the researcher to track movement of the tracer through the system over time but  
69 does not alter the soil C cycle's function. Radiocarbon ( $^{14}\text{C}$ ) has been a commonly used tracer  
70 across a myriad of systems and scales since the bomb spike was discovered in the 1960's  
71 (Cook et al., 2009; Harkness, 1970), and its utility with respect to understanding C cycle  
72 dynamics in the pedosphere was soon leveraged in a variety of soil C studies (Harkness et al.,  
73 1986; O'brien and Stout, 1978). However, the spike is limited in both magnitude and time scale.  
74 The bomb spike roughly doubled the amount of radiocarbon in the atmosphere by 1967, but  
75 afterwards the spike rapidly declined, making clear detection of the uptake of the bomb spike  
76 among pools and with depth difficult to detect in many soils. Additionally, multiple time points  
77 are required to estimate transfer rates and patterns, necessitating multiple sampling events  
78 many years apart, or the existence of archived samples.

79 The Enriched Background Isotope Study (EBIS) on the Oak Ridge Reservation in  
80 Tennessee, USA offered a unique opportunity to utilize a large and accidental release of  $^{14}\text{C}$   
81 radiocarbon tracer. In 1999, a large release of  $^{14}\text{C}$ -enriched  $\text{CO}_2$  from a local incinerator resulted  
82 in labeling of the surrounding forests (Trumbore et al., 2002), creating an extremely rare whole  
83 system tracer experiment. Following discovery of the event, researchers launched a series of  
84 investigations into the terrestrial C cycle including examination of soil respiration, dissolved C,  
85 microbial biomass, forest floor, roots, mycorrhizae, and mineral soils (Kramer et al., 2010;  
86 Swanston et al., 2005; Tipping et al., 2012; Treseder et al., 2006), building upon existing  
87 understanding of C cycling at this long-term ecosystem study site (Curtis et al., 2002; Davidson  
88 et al., 2002; Hanson et al., 1993; Johnson and Van Hook, 2012). This labeling event was similar  
89 in magnitude to the bomb spike, but highly localized. Early detection of the labeling event  
90 allowed for the installation of litter-swap treatments between labeled and unlabeled sites. This

91 litter-swap manipulation allowed for comparison of the relative importance of roots versus leaf  
92 litter as a source of C to soils on a yearly basis. We used measurements of radiocarbon  
93 abundance of organic horizons and mineral soils over a period of four years to gain insight into  
94 the sources, pathways, and stability of distinct pools of soil C with a specific focus on the  
95 following questions: 1) In this temperate forest ecosystem, what is the largest source of C to soil:  
96 litter or roots? 2) Are there significant differences in annual C partitioning among different  
97 pools/fractions of SOC? 3) Specifically, how is tracer transferred among fractions? 4) How  
98 quickly does newly introduced C propagate to depth? and, 5) How homogeneous are C uptake  
99 rates within the mineral-associated pool/fraction of SOC?

100

## 101 **METHODS:**

102

### 103 ***Site and experiment description***

104 In 2001, sixteen 7x7 m experimental plots were established at two sites on the Oak  
105 Ridge Reservation: Tennessee Valley Authority and Walker Branch (Swanston et al., 2005;  
106 Trumbore et al., 2002) (Figure 1, Supp. Fig. 1). The sites are very similar in all ecosystem  
107 properties, including topography (upper slope and ridge positions), parent material (dolomitic  
108 and cherty limestone residuum), vegetation (dominantly *Quercus* spp. and *Acer rubrum*., with  
109 *Carya* spp., *Pinus echinata*, *P. virginiana*, *Liriodendron tulipifera*, *Fagus grandifolia* and other  
110 accessory taxa) and soils (Fullerton series Typic Paleudults (USDA classification); Haplic Alisol  
111 (WRB classification) with clay phases dominated by kaolinite and hydroxy-Al interlayered  
112 vermiculite). Sand/silt/clay percentages ranged from 36/62/2 in the uppermost surface layer to  
113 12/49/39 in the deepest layer. No carbonates were present in the soils, as pH values ranged  
114 from 5.0 in the surface to 3.9 at depth (Peters et al., 1970).

115 In 1999, the Tennessee Valley Authority site was exposed to very high levels of  $^{14}\text{CO}_2$ ,  
116 and thereby the trees at this site incorporated a large amount of  $^{14}\text{C}$  label. Because the trees at

117 Tennessee Valley Authority incorporated high levels of  $^{14}\text{CO}_2$  into their photosynthates, the leaf  
118 litter and roots they produced were also highly labeled. The enriched litter at Tennessee Valley  
119 Authority  $\Delta^{14}\text{C}$  values were  $\sim 1000\text{‰}$ , while the enriched roots varied from 261-455‰ (Fröberg et  
120 al., 2007; Joslin et al., 2006). Walker Branch was exposed to a much lower and shorter pulse of  
121  $^{14}\text{CO}_2$ . Therefore, even though tree rings show some incorporation of  $^{14}\text{C}$  label at this site, levels  
122 are far below that of Tennessee Valley Authority, and the litter and roots they produced were  
123 near ambient atmospheric values at that time (litter  $\sim 221\text{‰}$ , roots  $\sim 125\text{-}294\text{‰}$ ). Employing a  
124 leaf litter replacement approach between the two sites, a total of four treatments were  
125 established across the two sites with four replicates each, yielding sixteen experimental plots  
126 total. Litter for the multi-year litter replacement study was collected in the fall of 2000 on Walker  
127 Branch (ambient litter) and Pine Ridge across the valley from the Tennessee Valley Authority  
128 site (enriched litter). Enough litter was collected in 200 to provide material for three years of  
129 subsequent treatment applications. During litter replacements over the following three years  
130 (March of 2001, January/February 2002 and 2003) approximately 500 g dry matter  $\text{m}^{-2}$  of the  
131 respective litter types were added to each of the plots, corresponding to the average annual  
132 litterfall at the sites. Each fall, natural litterfall for each plot fell onto a 7x7 m section of landscape  
133 cloth allowing for its removal and replacement by the target litter types. At Walker Branch, the  
134 treatments included control plots with ambient litter inputs (*control treatment*; replacement  
135 ambient leaf litter and natural root turnover) and enriched foliar litter plots with natural root  
136 turnover (*litter treatment*). At Tennessee Valley Authority, the treatments included a roots only  
137 enriched plots with ambient foliar litter (*roots treatment*), and enriched foliar litter and root input  
138 (*roots+litter treatment*) (Supplementary figure 1).

139 It is important to note that the application of labeled and near-background litter at the  
140 *litter* and *roots* treatment plots was instigated in March of 2001, approximately one and a half  
141 years after the release of the  $^{14}\text{CO}_2$  plume at Tennessee Valley Authority which occurred  
142 between July and August of 1999 (Trumbore et al., 2002). From the time of the  $^{14}\text{CO}_2$  release in

143 1999, <sup>14</sup>C label was actively incorporated into root material at Tennessee Valley Authority  
144 though leaves did not show evidence of strong incorporation of the label until formation of new  
145 buds in spring of 2000 (Trumbore et al., 2002). Therefore, introduction of <sup>14</sup>C label from root  
146 exudates and turnover at Tennessee Valley Authority was occurring 1-2 years prior to possible  
147 introduction of <sup>14</sup>C label from applied leaf litter at the *litter* and *roots+litter* treatments. Analysis of  
148 tree rings also indicated additional lower-level unquantified enrichment events over time at  
149 Tennessee Valley Authority (Trumbore et al., 2002). Those events increased the treatment  
150 effect at Tennessee Valley Authority, especially those associated with root inputs.

151

### 152 ***Soil and litter sample collection***

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

164

### 165 ***Soil analysis***

166 Soils from the 0-15 cm and 15-30 cm depths were density separated into free light  
167 fractions (FLF), occluded light fractions (OLF) and heavy fractions (HF) using a sodium

168 polytungstate solution adjusted to a density of  $1.70 \text{ g cm}^{-3}$  and sonication to disrupt aggregates  
169 ( $200 \text{ J mL}^{-1}$ ) (Golchin et al., 1994; Swanston et al., 2005). Samples were ground prior to further  
170 analysis. A selected subset of heavy fractions from 2004 was further density separated into a  
171 fraction with a density range of  $1.7\text{-}2.4 \text{ g cm}^{-3}$  and a fraction with a density  $>2.4 \text{ g cm}^{-3}$ , with the  
172 goal of investigating the level of homogeneity in incorporation of label in the heavy fraction. A  
173 separation at  $2.4 \text{ g cm}^{-3}$  was chosen in an effort to separate the heavy fraction into a fraction  
174 rich in phyllosilicate clays and a denser fraction rich in quartz. Only samples from 2004 from the  
175 *control*, *litter*, and *roots* treatments were density separated into  $1.7\text{-}2.4$  and  $>2.4 \text{ cm}^{-3}$  fractions.  
176 Due to the time and expense associated with these additional density separations, only a small  
177 subset of the heavy fractions could be examined. Samples from the 2004 were chosen since  
178 these samples represented the longest exposure time since labeling and therefore were  
179 hypothesized to show the largest treatment effect.

180 Total nitrogen (N) and C concentrations, and stable C and N isotope analyses of bulk  
181 soils and soil fractions were performed at Lawrence Berkeley National Laboratory. Samples  
182 were ground, powdered, and aliquots were loaded in Sn capsules for analysis. The capsules  
183 were then loaded into a zero blank autosampler connected to an ECS 4010 Elemental Analyzer  
184 (Costech Analytical Technologies Inc., Valencia, USA) coupled to a Delta V<sup>plus</sup> isotope ratio  
185 mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). Based on laboratory internal  
186 standards, the analytical precision on concentrations are  $\pm 0.05 \%$  weight ( $1\sigma$ ) for nitrogen,  $\pm$   
187  $0.59 \%$  weight ( $1\sigma$ , where  $\sigma$  is standard deviation) for carbon, for  $\delta^{13}\text{C}_{\text{VPDB}} \pm 0.21\text{‰}$  ( $1\sigma$ ), and for  
188  $\delta^{15}\text{N}_{\text{AIR}} \pm 0.32\text{‰}$  ( $1\sigma$ ).

189 Samples were graphitized in preparation for  $^{14}\text{C}$  abundance measurement at the Center  
190 for Accelerator Mass Spectrometry at Lawrence Livermore National Laboratory. Following  
191 sieving, samples were dried, weighed into quartz tubes along with silver and cupric oxide, and  
192 sealed under vacuum. Samples were combusted at  $900^\circ\text{C}$  for 6 hours to form  $\text{CO}_2$  gas. The

193 CO<sub>2</sub> was then reduced to graphite through heating at 570°C in the presence of hydrogen (H<sub>2</sub>)  
194 gas and an iron (Fe) catalyst (Vogel et al., 1987). Graphite targets were then analyzed for  
195 radiocarbon abundance (Davis et al., 1990), and corrected for mass-dependent fractionation  
196 following (Stuiver and Polach, 1977). Radiocarbon measurements were conducted on all free  
197 light, occluded light, and heavy fractions, bulk soils, as well as the 1.7-2.4 and >2.4 fractions.

198

### 199 ***Statistical approach***

200 Our statistical approach tested for the effect of treatment (root versus litter) over time,  
201 with depth, and among density fractions. Specifically, we looked for evidence of uptake of label  
202 from either roots or litter to the surrounding soil. Due to differences in length and degree of  
203 exposure to the labeling event, Walker Branch and Tennessee Valley Authority were analyzed  
204 separately. Variance in radiocarbon abundance values ( $\Delta^{14}\text{C}$ ) in fractions within each site was  
205 assessed using the GLIMMIX procedure and a repeated measures split-split-plot design with a  
206 normal distribution and an identity link function using SAS 9.4 (Cary, 2011). Treatment, depth,  
207 year, fraction and their interaction terms were tested for significance as fixed effects. Plot,  
208 plot\*average depth, and plot\*average depth\*fraction were the random effects in the model. To  
209 assess the correlation between years, an auto-regressive order 1 covariance structure was  
210 used, and a Kenward Rogers denominator degrees of freedom adjustment was applied.

211 Residuals were evaluated for homogeneity of variance and normality and were adjusted to  
212 include a heterogeneous covariance structure or using the group=option when needed. Tukey-  
213 Kramer Least Squares Means Adjustment for Multiple Comparisons was used to test for  
214 significant differences among treatments, depths, years, fractions, and their associated  
215 interactions for significant fixed effects. Variances in bulk  $\Delta^{14}\text{C}$  and fraction  $\Delta^{14}\text{C}$  values were  
216 assessed in two separate sets of models. One set included data associated with the free light,  
217 occluded light, and heavy fractions, the other set included only the bulk soil values. Variance in  
218 radiocarbon abundance values ( $\Delta^{14}\text{C}$ ) in bulk soils within each site was modelled under the

219 same conditions as the fractions, but with a repeated measures split-plot design. Due to the  
220 limited size of the sample set, 1.7-2.4 and >2.4 g cm<sup>-3</sup> fractions were only compared using a  
221 series of paired *t*-tests. Pearson correlation coefficients of a linear relationship were calculated  
222 to assess the presence and strength of associations among free light, occluded light and heavy  
223 fractions, and bulk soils.

#### 224 ***Estimation of turnover times***

225 We estimated the turnover time of organic C from the 0-15 cm layer of soil at the ORR in  
226 two ways. First, we used radiocarbon data from density fractionations of soils from the ambient  
227 treatment of the Throughfall Displacement Experiment which was carried out in plots adjacent to  
228 the current experiment (Hanson and Wullschleger, 2003). Soils were sampled prior to the <sup>14</sup>CO<sub>2</sub>  
229 labeling event, allowing for the estimation of a steady state mean residence time based on  
230 natural abundance radiocarbon measurements (Torn et al., 2002; Trumbore, 1993). Second, we  
231 examined the average rate of incorporation of the <sup>14</sup>CO<sub>2</sub> label in the soils from 0-15 cm at the  
232 *roots* treatment plots. Because we did not have soil samples from the *roots* treatment plots prior  
233 to the labeling event, we compared the average radiocarbon values of the soils at the *roots*  
234 treatment in 2001 to the average radiocarbon values of the soils at the *control* treatment in  
235 2001. We used a two-pool mixing model to assess the amount of new inputs into each fraction  
236 over a one-and-a-half-year period (i.e. label event happened in August, 1999, soils were  
237 sampled in March, 2001).

$$238 \quad D_c P_c + D_r P_r = D_f$$

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

#### 244 **RESULTS:**

245

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

### 252 ***Mass, C and N recovery***

253 Mass recovery following density fractionation averaged  $99.4 \pm 0.3\%$  at the Tennessee  
254 Valley Authority site, and  $100.0 \pm 0.1\%$  at the Walker Branch site. Recovery of C following  
255 density fractionation was more variable. At Tennessee Valley Authority, C recovery averaged  
256  $93.0 \pm 1.0\%$ , with a maximum of 170% and a minimum of 58%. Walker Branch C recovery  
257 averaged  $101.1 \pm 2.6$ , with a maximum of 302% and a minimum of 71%. This variability in C  
258 recovery is not uncommon given the inherent heterogeneity in particulate organic matter  
259 distribution in soils and the variance associated with soluble C being lost during density  
260 separation (Crow et al., 2007). Variability in N recovery was very high, despite mass recovery  
261 values near 100%. This variability may be due to a combination of the low N concentrations of  
262 the soils and the low (in comparison to N concentrations) precision of the elemental analyzer.  
263 Following density separation into the 1.7-2.4 and  $>2.4 \text{ cm}^{-3}$  fractions, mass recovery ranged  
264 from 98-105% and averaged 100% of the starting heavy fraction mass. Recovery of C averaged  
265  $91\% \pm 7\%$  (1  $\sigma$ ) across both sites.

266

### 267 ***Bulk, free light fraction, occluded light fraction, heavy fraction: C and N contents,*** 268 ***distribution, $\Delta^{14}\text{C}$ values***

269 Percent total C values for bulk soils ranged from an average of 2.54% for surface soils  
270 (the 0-15 cm layer) to less than 1% for soils at depths of up to 1 m (Table 1, Supp. Fig. 2). Total

271 N concentration of bulk soil ranged from an average of 0.12% to 0.01% (Table 1, Supp. Fig. 3).  
272 Percent C values for free light fractions averaged ~30%, occluded light averaged ~40% and  
273 heavy fractions ~1%. The majority of soil C resided in the heavy fraction (58%), with lesser  
274 amounts distributed to the free light (~30%) and occluded light (~13%) fractions (Figure 2, Table  
275 2, Supp. Fig. 4; note the high degree of variability in C distribution across time which is evident  
276 in all treatments). Percent N values for free light fractions averaged ~0.8%, occluded light  
277 averaged ~0.9% and heavy fractions ~0.05% (Supp. Fig. 5). When recalculated to sum to  
278 100%, the majority of the N resided in the heavy fraction (83%), followed by the free/light  
279 fraction (12%), and the occluded/light fraction (5%) (Supp. Fig. 6).

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

291 Only soils from the 0-15 and 15-30 cm depth increments were density separated and  
292 measured for  $\Delta^{14}\text{C}$ . In general, for all treatments and sites,  $\Delta^{14}\text{C}$  values of the three primary  
293 density fractions followed the pattern free light > occluded light > heavy, and all fractions  
294 declined in  $\Delta^{14}\text{C}$  value with increasing depth (Fig. 4). As was observed in bulk  $\Delta^{14}\text{C}$  values,  
295 averaged fraction  $\Delta^{14}\text{C}$  data also grouped by site, with Tennessee Valley Authority exhibiting  
296 elevated  $\Delta^{14}\text{C}$  values in comparison to Walker Branch (Table 4).

297

298 ***Effect of treatment and time in fractions***

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

304 A comparison of the  $\Delta^{14}\text{C}$  values of the free light, occluded and heavy fractions at the  
305 two treatment installments at the Tennessee Valley Authority site indicated that treatment was  
306 not a significant effect ( $p=0.8718$ ). The  $\Delta^{14}\text{C}$  values of *roots* and *roots+litter* treatments did vary  
307 as a function of depth ( $p<0.0001$ ), fraction ( $p<0.0001$ ), year\*fraction ( $p<0.0001$ ), and year\*depth  
308 ( $p=0.0247$ ). As evidenced by differences in p-values associated with individual fixed factors, in  
309 all cases, the significance of the interaction terms was driven by depth and fraction, with  
310 treatment and year having very little influence in the interaction. Across all years, fractions from  
311 0-15 cm were significantly enriched in  $\Delta^{14}\text{C}$  in comparison to fractions from 15-30 cm of depth  
312 ( $p<0.0001$  for all comparisons). Across years, the free light fraction was always enriched in  
313 comparison to heavy and occluded light fractions ( $p<0.0001$  for all comparisons). Heavy and  
314 occluded light fractions varied in their relationship over time. The four-way interaction term in the  
315 model comparing *control* and *litter* is most likely the product of increasing occluded light  $\Delta^{14}\text{C}$   
316 values in the *litter* treatment over time (Fig. 5). A summary of fixed effect statistics is given in  
317 supplementary table 1.

318 Pearson correlation coefficients were calculated for pairwise comparisons of fractions  
319 and bulk soil  $\Delta^{14}\text{C}$  values, shown as density ellipse fits (Fig. 5). Coefficients were interpreted as  
320 expressing either a strong ( $r>0.70$ ), moderate ( $r=0.50-0.69$ ), weak ( $r=0.30-0.49$ ), or very weak  
321 ( $r<0.29$ ) relationship between the two variables under comparison. Heavy and free light

322 fractions were strongly related to each other and bulk soil. In contrast, occluded fractions were  
323 only weakly or very weakly related to heavy and free light fractions and bulk soils.

324

### 325 ***Effect of treatment and time in bulk soils***

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

334

### 335 ***Differences in incorporation of label in 1.7-2.4 g cm<sup>-3</sup> and >2.4 cm<sup>-3</sup> fractions***

336 A comparison of 1.7-2.4 and >2.4 fractions across treatments and depths yielded several  
337 interesting differences (Table 5). The 1.7-2.4 fraction accounted for 4% (+/- 3% standard  
338 deviation) of the overall heavy fraction mass. The >2.4 fraction accounted for 96% (+/- 4%  
339 standard deviation) of the overall heavy fraction mass. C concentrations of 1.7-2.4 fractions  
340 were two orders of magnitude larger than C concentrations of the >2.4 fraction (14.21% vs.  
341 0.37%). Though the masses of the two fractions were very different, total C was more-or-less  
342 evenly distributed between the 1.7-2.4 and >2.4 fractions, at 55% +/- 16% and 45% +/-16%,  
343 respectively (Table 5). N concentrations were 20 times higher in the 1.7-2.4 fractions as in the  
344 >2.4 fractions (0.60% vs. 0.03%). Mean C/N molar ratios were significantly lower in the >2.4  
345 fraction (14 vs. 28), and  $\delta^{13}\text{C}$  values were significantly higher ( $\sim -25\text{‰}$  vs.  $\sim -27\text{‰}$ ).

346 A statistical comparison of the 1.7-2.4 and >2.4 fractions among the three treatments  
347 could not be made, due to differences in the initial plume strength at the two sites. A qualitative

348 comparison, however, did suggest some trends. Values of  $\Delta^{14}\text{C}$  appear to be elevated in the  
349 *roots* treatment in comparison to the *control* and *litter* treatments (Figure 7, Supp. Table 3). The  
350 combined 1.7-2.4 and >2.4 fractions from *Control* and *litter* treatments were compared using a  
351 simple t test and were found to not be different at the  $\alpha = 0.05$  level ( $p = 0.7436$ ; i.e. no litter  
352 treatment effect). The  $\Delta^{14}\text{C}$  values of the 1.7-2.4 and >2.4 fractions show a nearly one-to-one  
353 positive relationship with each other. In the *roots* treatment, the uptake of label and the relative  
354 increase in  $\Delta^{14}\text{C}$  was similar in both fractions.

### 355 *Estimates of turnover times*

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

### 364 ***Compilation of current and prior measurements***

365 A substantial number of studies have utilized the EBIS experimental site and/or  
366 materials taken from the site. These studies focused on different components of the ecosystem  
367 such as roots, microbial biomass, DOC, and soil respired  $\text{CO}_2$  (references given in  
368 Supplementary table 4). In order to examine and illustrate possible connections among different  
369 C cycle pools, we present compiled illustrations of C stocks,  $\Delta^{14}\text{C}$ , and hypothetical connections  
370 among the measured pools (Supplementary figures 7-11).

### 371 **DISCUSSION:**

372

373 *In this temperate forest ecosystem, what is the largest source of C to soil: litter or roots?*

374 This unique whole-system labelling experiment indicates that roots are the primary  
375 source of mineral soil C, while contributions of leaf litter to subsoil C pools are minimal. Though  
376 percolation of litter-derived dissolved phase C may contribute significantly to soil C stocks in  
377 some systems, such as northern hardwood forests (Rothstein et al., 2018), only a small uptake  
378 of tracer by dissolved organic C was detected in the *litter* treatment (Fröberg et al., 2007).  
379 Results from both bulk soils and soil fractions support the conclusion that roots are the source of  
380 the majority of C in mineral soils at these sites, as has been suggested by previous work in  
381 these and other soils (Kramer et al., 2010; Liebmann et al., 2020; Pausch and Kuzyakov, 2018).  
382 Though explicit statistical comparisons cannot be drawn between Walker Branch and  
383 Tennessee Valley because of variable exposures to the enrichment label, soil  $\Delta^{14}\text{C}$  values at  
384 Tennessee Valley can logically be assumed to have been highly similar to values at the Walker  
385 Branch *control* treatment prior to the labelling event in 1999 (Trumbore et al., 2002), and a  
386 qualitative comparison indicates large differences in  $\Delta^{14}\text{C}$  values of bulk soils and fractions  
387 between the two sites (Tables 3, 4, Figures 4, 5). Consistent ratios of root biomass to bulk C stocks,  
388 ranging from ~4-7 across the four sampled depth increments (Supp. Fig. 7) suggest inputs are at steady  
389 state, but with total inputs, input rates, and therefore bulk C stocks decreasing with depth. Radiocarbon  
390 abundance also decreases with depth, suggesting the rate of input must also be decreasing with depth.  
391 This is consistent with the idea that turnover of bulk SOC is directly related to the magnitude of  
392 fresh inputs (Fontaine et al., 2007).

393 Measures of microbial biomass and respired  $^{14}\text{CO}_2$  from previous publications (Cisneros-  
394 Dozal et al., 2006; Kramer et al., 2010) additionally suggest that roots are not only the main  
395 source of stabilized SOC but are also the main source of microbial substrate (Kramer et al.,  
396 2010). Microbial metabolism drives SOC turnover while also contributing to a significant pool of  
397 subsoil organic matter in the form of microbial necromass (Cotrufo et al., 2013). However,  
398 tracking the flow of substrate to and through microbial biomass is nontrivial (Dannenmann et al.,

399 2009; Kögel-Knabner, 2017). Here, differences in label uptake by microbes among treatments  
400 allows for identification of their main substrate sources. Based on  $^{14}\text{CO}_2$  measurements of  
401 incubated surface soils (0-5 cm, A horizon), after 4 years of labeled litter additions, only ~6% of  
402 microbial biomass was derived from labeled Oi carbon (Kramer et al., 2010). Additionally, the  
403  $\Delta^{14}\text{C}$  values of fine roots reported by Joslin et al. (2006) were found to closely resemble the  
404  $\Delta^{14}\text{C}$  values of the PLFA compounds extracted from soils at each site, further suggesting that  
405 roots are the main substrate for the soil microbial community (Supplementary figures 10, 11).

406 Results suggest a small but significant contribution of DOC from Oi layers to the  
407 subsurface C cycle. Contributions of DOC from newly added litter to mineral soils are evident  
408 based on DOC measurements from previous work (Fröberg et al., 2009; Fröberg et al., 2007).  
409 However, these DOC contributions were not significant enough to alter the  $\Delta^{14}\text{C}$  values of the  
410 bulk soils. The lack of change in bulk soil  $\Delta^{14}\text{C}$  combined with measurement of microbial  
411 biomass and soil respired  $\text{CO}_2$   $\Delta^{14}\text{C}$  values (Cisneros-Dozal et al., 2006; Kramer et al., 2010)  
412 (Supplementary figures 8-11) suggested that DOC was being almost wholly consumed as a  
413 microbial growth substrate. This is consistent with work illustrating the dynamic nature of the  
414 water-soluble fraction of SOC (van der Voort et al., 2019).

415 Radiocarbon label derived from labeled litter additions (presumably in the form of labeled  
416 DOC) did not result in a treatment effect in the bulk soils of the *control* vs. *litter* treatments at  
417 Walker Branch but did result in a treatment effect in the bulk soils between *roots* and *roots+litter*  
418 treatments at Tennessee Valley (Supp. Table 2). This is most likely the result of differences in  
419 the timing of the implementation of the treatments at the two sites. Tennessee Valley was  
420 receiving an unknown amount of tracer 1-2 years prior to installation of treatments at Walker  
421 Branch, therefore labeled DOC may have had a substantially longer period of time to  
422 translocate to depth in the *roots+litter* treatment than in the *litter* treatment. This conclusion is  
423 supported by the significance of year\*depth in the bulk soils model at Tennessee Valley, which  
424 was not significant at Walker Branch. Ultimately, though, this statistical significance highlights

425 that while our experiment was able to detect an isotopic signal of DOC translocation, it only  
426 indicates that this process is occurring and does not address the quantity of C that was  
427 translocated vertically. Earthworms have been shown to affect label movement in similar  
428 studies, as some species harvest leaf litter and transport the material to depth (McFarlane et al.,  
429 2013; Wenk et al., 2016). In the current study, we are confident in assigning vertical transport of  
430 label to the movement of dissolved organics as opposed to bioturbation, as there is a clear  
431 distinction between litter layers of differing decomposition status, and a clear abrupt boundary at  
432 the organic/mineral soil interface.

433

434 *Are there significant differences in annual C uptake among different pools/fractions of SOC?*

435 *How quickly does newly introduced C propagate to depth? How deep does it go?*

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

447 Historical (Golchin et al., 1994; Tipping et al., 2012) models once postulated a passage  
448 of new inputs from the free light through the occluded and into the mineral-stabilized heavy  
449 fraction. The current results show that in A and E horizons (the 0-15 cm sampling layer), root-  
450 derived C is introduced to both the free light and heavy fractions simultaneously, and SOC does

451 not appear to pass through the occluded light fraction into the heavy fraction, as evidenced by  
452 an immediate incorporation of label into the heavy fraction and a delayed incorporation of label  
453 into the occluded light fraction which appeared to increase over time (Figure 5). More recent  
454 conceptual models suggest a tightly-bound inner layer of organics directly associated with  
455 mineral surfaces, coated with increasing exchangeable layers of organics as distance from the  
456 mineral surface increases (i.e. the “onion model” (Kleber et al., 2007; Sollins et al., 2006)). This  
457 previous work illustrated a steady decline in radiocarbon abundance with increasing particle  
458 abundance across a variety of soils, suggesting that sequentially separating soils into denser  
459 and denser fractions allows for the partitioning of mineral-associated organics into fractions with  
460 increasingly thinner organic matter coatings and slower turnover as density increases (Sollins et  
461 al., 2009). Based on these observations, we expected the densest fraction ( $>2.4 \text{ g cm}^{-3}$ ) to have  
462 the thinnest, most tightly-bound organic layer which would also be the most depleted in  
463 radiocarbon, but this was not the case. Not only was the  $>2.4$  fraction enriched in radiocarbon in  
464 comparison to the 1.7-2.4 fraction in the 0-15 cm depth, it also incorporated more label than the  
465 1.7-2.4 fraction in the *roots+litter* treatment. This substantial uptake of label in the densest  
466 portion of the mineral-associated fraction in this study ( $>2.4 \text{ g cm}^{-3}$ ) suggests that all organics  
467 from all densities and degrees of mineral association have a significant portion of C which  
468 cycles on a near-annual basis. This is additional empirical evidence of the heterogeneous  
469 nature of SOC fractions and suggests that regardless of how finely partitioned (sequentially  
470 density separated) a soil is partitioned, fractions may always contain a substantial portion of C  
471 with a fast turnover.

472         Given that roots are the source of the majority of SOC, and that changes in the  $\Delta^{14}\text{C}$   
473 values of fractions are indicative of an incorporation of root-derived labeled C, this simple rate of  
474 transfer can be used to estimate turnover of C in each fraction. This approach yielded mean  
475 residence times of 2, 4, and 18 years for free light, heavy and occluded fraction from the 0-15  
476 cm depth. Additionally, incorporation rates associated with the uptake of tracer indicate that

477 substantial amounts of new organics are incorporated into fractions on a yearly basis (79% of  
478 total C in the free light, 8% in the heavy, and 37% in the occluded). These timespans are very  
479 short in comparison to steady state mean residence times calculated from natural abundance  
480  $\Delta^{14}\text{C}$  values taken from unlabeled soil near the two sites (Johnson et al., 2008) (0-15 cm), and  
481 using a steady-state mean residence time model (Torn et al., 2002; Trumbore, 1993), which  
482 averaged 37, 108, and 71 years for free light, heavy, and occluded fractions. Both calculations  
483 indicate differences in the annual incorporation of new C by the individual fractions, however the  
484 two estimations agree neither in magnitude nor pattern. This discrepancy among methods for  
485 estimating SOC turnover is well documented in (Feng et al., 2016), which used a meta-analysis  
486 of 52 studies to examine how mean residence time estimations vary according to methodology.  
487 Their analysis indicated that estimates based on natural abundance radiocarbon values were  
488 consistently 2-4 times greater than estimates based on C3/C4 vegetation switches and up to an  
489 order of magnitude greater than estimates derived from soil incubations. We speculate that the  
490 discrepancies in our own dataset result from non-homogeneity within each of the density  
491 fractions, with a portion of each experiencing very rapid turnover and other portions having  
492 much longer mean residence times. This proposition is strongly supported by the consistent and  
493 significant differences in composition and radiocarbon abundance between the 1.7-2.4 g cm<sup>-3</sup>  
494 and >2.4 g cm<sup>-3</sup> fractions (Table 5, Figure 7).

495         The influence of time on the transfer of label to depth was not immediately interpretable.  
496 Treatment, and year\*depth were both significant effects in the Tennessee Valley Authority bulk  
497 soil model (Supp table 2), with the *roots+litter* treatment incorporating more label relative to the  
498 *roots* treatment. However, changes in  $\Delta^{14}\text{C}$  values over time fluctuated instead of increasing  
499 steadily over time (Figure.3) Label was detected at up to 45 cm of depth in the Tennessee  
500 Valley plots at the time of the first sampling, though was never detected below this depth even  
501 after 4 years of label incorporation, suggesting a very slow rate of incorporation of new  
502 photosynthates in deep soils during this experiment. The rate of label incorporation decreased

503 with increasing depth in general, which is consistent with decreases in annual root inputs with  
504 increasing depth (Joslin et al., 2006).

505

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

508 Previous work utilizing natural abundance radiocarbon measurements indicate that free  
509 light fractions cycle much more rapidly than heavy fractions. However, this whole system  
510 labelling experiment indicates that free light and heavy fractions share the same pathways of  
511 incorporation of newly fixed photosynthates, namely direct annual input from roots, whether as  
512 particulate litter (FLF) or molecular-scale residues and exudates (HF). This is further illustrated  
513 by strong correlations among free light and heavy fraction  $\Delta^{14}\text{C}$  values (Figure 5). These  
514 relationships are absent in the occluded light  $\Delta^{14}\text{C}$  values which show only very weak  
515 relationships with other fractions or bulk  $\Delta^{14}\text{C}$  values. Therefore, our data suggests occluded  
516 light fraction incorporates C from the free light and heavy fractions over a multi-year time span,  
517 which we hypothesize occurs through the process of aggregate turnover. This is consistent with  
518 more contemporary modeling efforts where C is incorporated into aggregates from both  
519 particulate and mineral-associated pools (Abramoff et al., 2018). We observed a strong  
520 relationship between heavy fraction and bulk soil  $\Delta^{14}\text{C}$  values, emphasizing the strong  
521 dependence of bulk C turnover rates on the mineral-associated pool which often comprises the  
522 majority of C in mineral soils, e.g.(Swanston et al., 2005).

523 Based on previous work with sequential density separation (see “onion model” above),  
524 we expected the further partitioning of the heavy fraction into the sub-fractions of differing  
525 density (1.7-2.4 g cm<sup>-3</sup> and >2.4 g cm<sup>-3</sup>) to result in isolation of an “inert” densest fraction (>2.4 g  
526 cm<sup>-3</sup>). Characteristics of the >2.4 cm<sup>-3</sup> fraction suggest a higher degree of microbial processing  
527 than the 1.7-2.4 cm<sup>-3</sup> fraction (i.e. narrowed C/N in combination with higher  $\delta^{13}\text{C}$  values, Table  
528 5), consistent with previous observations (Kleber et al., 2011; Sollins et al., 2009; Sollins et al.,

529 2006). This pattern of increasing degree of microbial processing with increasing heavy fraction  
530 density would seem to suggest differences in rates and pathways of incorporation of C into  
531 these sub-fractions. However, both of these fractions indicate incorporation of label in the *roots*  
532 treatment, and that this incorporation is of similar magnitude. Similar rates of incorporation of  
533 label in combination with differing degrees of microbial processing would seem to be  
534 contradictory. Again, we interpret this discrepancy as evidence of non-homogeneity within each  
535 of these sub-fractions, with each sub-fraction containing portions of faster- and more slowly-  
536 cycling organics.

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

543

544 *How do these results fit into an ecosystem context?*

545         This experiment was conducted at a site that is arguably one of the best-studied forest  
546 landscapes in the literature. Studies most relevant to the current study address C and nutrient  
547 stocks in experimental harvests (e.g. Johnson et al. 1988; Johnson and Todd 1998),  
548 precipitation manipulations (e.g., Johnson et al. 2008; Fröberg et al. 2008) and untreated control  
549 forests that span a wide range of parent materials, geomorphic settings, and soil and  
550 vegetation types (Trettin et al. 1999). Our study was conducted in typical residual Ultisols  
551 formed in the limestone and dolomite saprolite of upper slope and ridge positions, under the  
552 most widely distributed, oak-dominated cover type at ORR (Trettin et al. 1999). This suggests  
553 that the soil C dynamics we report here are representative of much of the local landscape, and

554 transferable to sites with similar physiography (well-drained, weathered uplands) across the  
555 wider Appalachian Ridge and Valley province.

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

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

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

578 **SUMMARY**

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

589

## 590 **ACKNOWLEDGEMENTS**

591 EBIS project participants appreciate access and use of Tennessee Valley Authority (TVA) land  
592 on Chestnut Ridge near the Oak Ridge Reservation allowed under Contract No. 105906  
593 between TVA and the Oak Ridge National Laboratory. Funding for the EBIS project was  
594 provided by the U.S. Department of Energy, Office of Science, Biological and Environmental  
595 Research (BER), as a part of the Terrestrial Carbon Processes (TCP) Program. We extend  
596 special thanks to Paula Zermeño for her expertise in the laboratory and the graphitization of the  
597 >600 radiocarbon samples included in this work.

598

## 599 **REFERENCES**

600

- 601 Abramoff, R., Xu, X., Hartman, M., O'Brien, S., Feng, W., Davidson, E., Finzi, A., Moorhead, D.,  
602 Schimel, J., Torn, M., 2018. The Millennial model: in search of measurable pools and  
603 transformations for modeling soil carbon in the new century. *Biogeochemistry* 137(1),  
604 51-71.
- 605 Bird, J.A., Kleber, M., Torn, M.S., 2008.  $^{13}\text{C}$  and  $^{15}\text{N}$  stabilization dynamics in soil organic  
606 matter fractions during needle and fine root decomposition. *Organic Geochemistry* 39(4),  
607 465-477.

608 Bradford, M.A., Wieder, W.R., Bonan, G.B., Fierer, N., Raymond, P.A., Crowther, T.W., 2016.  
609 Managing uncertainty in soil carbon feedbacks to climate change. *Nature Climate*  
610 *Change* 6(8), 751-758.

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

613 Cisneros-Dozal, L.M., Trumbore, S., Hanson, P.J., 2006. Partitioning sources of soil-respired  
614 CO<sub>2</sub> and their seasonal variation using a unique radiocarbon tracer. *Global Change*  
615 *Biology* 12(2), 194-204.

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

618 Cotrufo, M.F., Wallenstein, M.D., Boot, C.M., Denef, K., Paul, E., 2013. The Microbial E  
619 fficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition  
620 with soil organic matter stabilization: do labile plant inputs form stable soil organic  
621 matter? *Global Change Biology* 19(4), 988-995.

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

625 Curtis, P.S., Hanson, P.J., Bolstad, P., Barford, C., Randolph, J., Schmid, H., Wilson, K.B.,  
626 2002. Biometric and eddy-covariance based estimates of annual carbon storage in five  
627 eastern North American deciduous forests. *Agricultural and Forest Meteorology* 113(1-  
628 4), 3-19.

629 Dannenmann, M., Simon, J., Gasche, R., Holst, J., Naumann, P.S., Kögel-Knabner, I., Knicker,  
630 H., Mayer, H., Schloter, M., Pena, R., 2009. Tree girdling provides insight on the role of  
631 labile carbon in nitrogen partitioning between soil microorganisms and adult European  
632 beech. *Soil Biology and Biochemistry* 41(8), 1622-1631.

633 Davidson, E.A., Savage, K., Bolstad, P., Clark, D.A., Curtis, P.S., Ellsworth, D.S., Hanson, P.J.,  
634 Law, B.E., Luo, Y., Pregitzer, K.S., 2002. Belowground carbon allocation in forests  
635 estimated from litterfall and IRGA-based soil respiration measurements. *Agricultural and*  
636 *Forest Meteorology* 113(1-4), 39-51.

637 Davis, J., Proctor, I., Southon, J., Caffee, M., Heikkinen, D., Roberts, M., Moore, T., Turteltaub,  
638 K.W., Nelson, D., Loyd, D., 1990. LLNL/UC AMS facility and research program. *Nuclear*  
639 *Instruments and Methods in Physics Research Section B: Beam Interactions with*  
640 *Materials and Atoms* 52(3-4), 269-272.

641 Feng, W., Shi, Z., Jiang, J., Xia, J., Liang, J., Zhou, J., Luo, Y., 2016. Methodological  
642 uncertainty in estimating carbon turnover times of soil fractions. *Soil Biology and*  
643 *Biochemistry* 100, 118-124.

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

646 Fröberg, M., Hanson, P.J., Trumbore, S.E., Swanston, C.W., Todd, D.E., 2009. Flux of carbon  
647 from <sup>14</sup>C-enriched leaf litter throughout a forest soil mesocosm. *Geoderma* 149(3-4),  
648 181-188.

649 Fröberg, M., Jardine, P.M., Hanson, P.J., Swanston, C., Todd, D., Tarver, J., Garten, C., 2007.  
650 Low dissolved organic carbon input from fresh litter to deep mineral soils. *Soil Science*  
651 *Society of America Journal* 71(2), 347-354.

652 Golchin, A., Oades, J., Skjemstad, J., Clarke, P., 1994. Study of free and occluded particulate  
653 organic matter in soils by solid state <sup>13</sup>C CP/MAS NMR spectroscopy and scanning  
654 electron microscopy. *Soil Research* 32(2), 285-309.

655 Griscom, B.W., Adams, J., Ellis, P.W., Houghton, R.A., Lomax, G., Miteva, D.A., Schlesinger,  
656 W.H., Shoch, D., Siikamäki, J.V., Smith, P., 2017. Natural climate solutions. *Proceedings*  
657 *of the National Academy of Sciences* 114(44), 11645-11650.

658 Hanson, P., Wullschleger, S., Bohlman, S., Todd, D., 1993. Seasonal and topographic patterns  
659 of forest floor CO<sub>2</sub> efflux from an upland oak forest. *Tree physiology* 13(1), 1-15.

660 Harkness, D., Harrison, A., Bacon, P., 1986. The temporal distribution of 'bomb'<sup>14</sup>C in a forest  
661 soil. *Radiocarbon* 28(2A), 328-337.

662 Harkness, D.D., 1970. Artificial carbon-14, a tracer for carbon in the atmosphere and biosphere,  
663 ProQuest Dissertations & Theses.

664 Johnson, D.W., Todd Jr, D.E., Hanson, P.J., 2008. Effects of throughfall manipulation on soil  
665 nutrient status: results of 12 years of sustained wet and dry treatments. *Global Change*  
666 *Biology* 14(7), 1661-1675.

667 Johnson, D.W., Van Hook, R.I., 2012. Analysis of biogeochemical cycling processes in Walker  
668 Branch Watershed. Springer Science & Business Media.

669 Joslin, J., Gaudinski, J.B., Torn, M.S., Riley, W., Hanson, P.J., 2006. Fine-root turnover patterns  
670 and their relationship to root diameter and soil depth in a <sup>14</sup>C-labeled hardwood forest.  
671 *New Phytologist* 172(3), 523-535.

672 Kleber, M., Nico, P.S., Plante, A., Filley, T., Kramer, M., Swanston, C., Sollins, P., 2011. Old  
673 and stable soil organic matter is not necessarily chemically recalcitrant: implications for  
674 modeling concepts and temperature sensitivity. *Global Change Biology* 17(2), 1097-  
675 1107.

676 Kleber, M., Sollins, P., Sutton, R., 2007. A conceptual model of organo-mineral interactions in  
677 soils: self-assembly of organic molecular fragments into zonal structures on mineral  
678 surfaces. *Biogeochemistry* 85(1), 9-24.

679 Kögel-Knabner, I., 2017. The macromolecular organic composition of plant and microbial  
680 residues as inputs to soil organic matter: fourteen years on. *Soil Biology and*  
681 *Biochemistry* 105, A3-A8.

682 Kramer, C., Trumbore, S., Fröberg, M., Dozal, L.M.C., Zhang, D., Xu, X., Santos, G.M., Hanson,  
683 P.J., 2010. Recent (< 4 year old) leaf litter is not a major source of microbial carbon in a  
684 temperate forest mineral soil. *Soil Biology and Biochemistry* 42(7), 1028-1037.

685 Liebmann, P., Wordell-Dietrich, P., Kalbitz, K., Mikutta, R., Kalks, F., Don, A., Woche, S.K.,  
686 Dsilva, L.R., Guggenberger, G., 2020. Relevance of aboveground litter for soil organic  
687 matter formation—a soil profile perspective. *Biogeosciences* 17(12), 3099-3113.

688 McFarlane, K.J., Torn, M.S., Hanson, P.J., Porras, R.C., Swanston, C.W., Callahan, M.A.,  
689 Guilderson, T.P., 2013. Comparison of soil organic matter dynamics at five temperate  
690 deciduous forests with physical fractionation and radiocarbon measurements.  
691 *Biogeochemistry* 112(1-3), 457-476.

692 Minasny, B., Malone, B.P., McBratney, A.B., Angers, D.A., Arrouays, D., Chambers, A.,  
693 Chaplot, V., Chen, Z.-S., Cheng, K., Das, B.S., 2017. Soil carbon 4 per mille. *Geoderma*  
694 292, 59-86.

695 O'brien, B., Stout, J., 1978. Movement and turnover of soil organic matter as indicated by  
696 carbon isotope measurements. *Soil Biology and Biochemistry* 10(4), 309-317.

697 Pausch, J., Kuzyakov, Y., 2018. Carbon input by roots into the soil: quantification of  
698 rhizodeposition from root to ecosystem scale. *Global Change Biology* 24(1), 1-12.

699 Paustian, K., Lehmann, J., Ogle, S., Reay, D., Robertson, G.P., Smith, P., 2016. Climate-smart  
700 soils. *Nature* 532(7597), 49-57.

701 Rasse, D.P., Rumpel, C., Dignac, M.-F., 2005. Is soil carbon mostly root carbon? Mechanisms  
702 for a specific stabilisation. *Plant and soil* 269(1-2), 341-356.

703 Rothstein, D.E., Toosi, E.R., Schaetzl, R.J., Grandy, A.S., 2018. Translocation of Carbon from  
704 Surface Organic Horizons to the Subsoil in Coarse-Textured Spodosols: Implications for  
705 Deep Soil C Dynamics. *Soil Science Society of America Journal* 82(4), 969-982.

706 Schmidt, M.W., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I.A., Kleber,  
707 M., Kögel-Knabner, I., Lehmann, J., Manning, D.A., 2011. Persistence of soil organic  
708 matter as an ecosystem property. *Nature* 478(7367), 49-56.

709 Sierra, C., Müller, M., Trumbore, S.E., 2014. Modeling radiocarbon dynamics in soils: SoilR  
710 version 1.1. *Geoscientific Model Development* 7(5), 1919-1931.

711 Sollins, P., Kramer, M.G., Swanston, C., Lajtha, K., Filley, T., Aufdenkampe, A.K., Wagai, R.,  
712 Bowden, R.D., 2009. Sequential density fractionation across soils of contrasting  
713 mineralogy: evidence for both microbial- and mineral-controlled soil organic matter  
714 stabilization. *Biogeochemistry* 96(1-3), 209-231.

715 Sollins, P., Swanston, C., Kleber, M., Filley, T., Kramer, M., Crow, S., Caldwell, B.A., Lajtha, K.,  
716 Bowden, R., 2006. Organic C and N stabilization in a forest soil: Evidence from  
717 sequential density fractionation. *Soil Biology and Biochemistry* 38(11), 3313-3324.

718 Spielvogel, S., Prietzel, J., Kögel-Knabner, I., 2016. Stand scale variability of topsoil organic  
719 matter composition in a high-elevation Norway spruce forest ecosystem. *Geoderma* 267,  
720 112-122.

721 Stuiver, M., Polach, H.A., 1977. Discussion reporting of  $^{14}\text{C}$  data. *Radiocarbon* 19(3), 355-363.

722 Swanston, C.W., Torn, M.S., Hanson, P.J., Southon, J.R., Garten, C.T., Hanlon, E.M., Ganio,  
723 L., 2005. Initial characterization of processes of soil carbon stabilization using forest  
724 stand-level radiocarbon enrichment. *Geoderma* 128(1-2), 52-62.

725 Tipping, E., Chamberlain, P.M., Fröberg, M., Hanson, P.J., Jardine, P.M., 2012. Simulation of  
726 carbon cycling, including dissolved organic carbon transport, in forest soil locally  
727 enriched with  $^{14}\text{C}$ . *Biogeochemistry* 108(1-3), 91-107.

728 Torn, M.S., Lapenis, A.G., Timofeev, A., Fischer, M.L., Babikov, B.V., Harden, J.W., 2002.  
729 Organic carbon and carbon isotopes in modern and 100-year-old-soil archives of the  
730 Russian steppe. *Global Change Biology* 8(10), 941-953.

731 Treseder, K.K., Torn, M.S., Masiello, C.A., 2006. An ecosystem-scale radiocarbon tracer to test  
732 use of litter carbon by ectomycorrhizal fungi. *Soil Biology and Biochemistry* 38(5), 1077-  
733 1082.

734 Trumbore, S., Gaudinski, J.B., Hanson, P.J., Southon, J.R., 2002. Quantifying  
735 ecosystem-atmosphere carbon exchange with a  $^{14}\text{C}$  label. *Eos, Transactions American*  
736 *Geophysical Union* 83(24), 265-268.

737 Trumbore, S.E., 1993. Comparison of carbon dynamics in tropical and temperate soils using  
738 radiocarbon measurements. *Global Biogeochemical Cycles* 7(2), 275-290.

739 Trumbore, S.E., Zheng, S., 1996. Comparison of fractionation methods for soil organic matter  
740  $^{14}\text{C}$  analysis. *Radiocarbon* 38(2), 219-229.

741 van der Voort, T.S., Mannu, U., Hagedorn, F., McIntyre, C., Walthert, L., Schleppei, P.,  
742 Haghypour, N., Eglinton, T.I., 2019. Dynamics of deep soil carbon—insights from  $^{14}\text{C}$   
743 time series across a climatic gradient. *Biogeosciences* 16(16), 3233-3246.

744 Vogel, J.S., Southon, J.R., Nelson, D.E., 1987. Catalyst and binder effects in the use of  
745 filamentous graphite for AMS. *Nuclear Instruments and Methods in Physics Research*  
746 *Section B: Beam Interactions with Materials and Atoms* 29(1-2), 50-56.

747 von Lützow, M., Kögel-Knabner, I., Ekschmitt, K., Flessa, H., Guggenberger, G., Matzner, E.,  
748 Marschner, B., 2007. SOM fractionation methods: relevance to functional pools and to  
749 stabilization mechanisms. *Soil Biology and Biochemistry* 39(9), 2183-2207.

750 Wagai, R., Mayer, L.M., Kitayama, K., 2009. Nature of the “occluded” low-density fraction in soil  
751 organic matter studies: A critical review. *Soil Science and Plant Nutrition* 55(1), 13-25.

752 Wenk, E.S., Callahan Jr, M.A., O'Brien, J.J., Hanson, P.J., 2016. Soil macroinvertebrate  
753 communities across a productivity gradient in deciduous forests of eastern North  
754 America. *Northeastern Naturalist* 23(1), 25-44.

755