Tracing carbon flow through a sugar maple forest and its soil components: role of invasive earthworms

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Abstract

Aims. We conducted a suite of tracer studies using the stable isotope ^{13}C to follow and quantify the flow of carbon from leaf litter and roots into soil components including aggregates and biota with and without invasive earthworms.

Methods. Ten-year-old saplings of sugar maple growing in the understory of a thinned northern hardwood forest were labeled with $^{13}CO_2$. The ^{13}C labeled leaf litter was applied to forest plots with and without invasive earthworms (Lumbricidae) and traced for three years. We also traced the label from the trees through the roots and into soil components in the labeling chambers. Labeled fine roots and stem wood were incubated in a forest and the label was quantified over six years of decomposition.

Results. We were able to detect the litter tracer to 10 cm soil depth in plots without

earthworms and to 20 cm with earthworms present, and earthworms promoted C incorporation into soil aggregates. The soil food web was much more enriched in the label from roots than from aboveground plant litter.. Rapid fine root decay was observed ($k=0.9\ yr^{-1}$), and although labelled wood was almost completely decayed, little ^{13}C was recovered in soil (0.33%).

Conclusion. The approach was successful for quantifying transport and fate of tree carbon in forest soils and could be enhanced with careful quantification of gross assimilation.

Keywords: aggregates, earthworms, litter decay, roots, salamander, stable isotope

Introduction:

Forest ecosystems play an important role in regulating the flow of carbon in the biosphere. Currently, temperate forests serve as a significant sink for atmospheric carbon, but the future of this ecosystem service depends upon a complex suite of forcings and feedbacks associated with anthropogenic activity. The pools of carbon in forest vegetation and soil are dynamic, and the processes influencing their turnover are not fully understood. In particular, the dynamics of forest soil carbon are highly complex, and a better understanding of the processes supplying forest SOM and its stabilization are subjects of great interest because of their potential to influence the global C budget. Forest ecosystem carbon dynamics can be regulated by global change drivers including land management activities. Hence, an improved understanding of the mechanisms of formation and stabilization of SOM is needed to inform C management activities.

A wide variety of approaches has been employed to study the forms and dynamics of forest SOM including, for example, biochemical and microscopic characterization, measuring microbial activity, simulation modeling and *in situ* ecosystem-scale mass-balance and tracer studies. The latter category includes the use of isotopes of carbon, radioactive ^{14}C and stable ^{13}C, to evaluate the formation, stability, and degradation of soil C. Radioactive ^{14}C methods have long been used to evaluate the age and dynamics of soil organic C (Sharpenseel et al. 1992), and the inadvertent addition of ^{14}C by atomic bomb testing in the atmosphere in the mid-20th century provided a valuable tool for studying soil organic carbon (Trumbore et al. 1989). The stable isotope ^{13}C has also been widely employed to study soil C; for example, field-scale tracing of

soil C dynamics has relied on the difference in ^{13}C natural abundance between plants using the C-4 vs the C-3 photosynthetic pathway (Balesdent et al. 1987), and more recently the in-growth soil core ^{13}C method has been effective for quantifying belowground C allocation (Martinez et al. 2016). Experiments employing laboratory-enriched ^{13}C compounds also are useful for quantifying soil carbon dynamics.

The stock of carbon in forest soils represents the long-term balance between plant production anddecomposition processes. The sources of forest SOM include aboveground and belowground detritus, rhizosphere carbon flux (RCF; exudation, rhizodeposition, mycorrhizal fungi) and by-products of microbial activity. The dominant role of belowground inputs, including both root detritus and RCF, in supplying forest SOM formation has been revealed by recent studies using various biochemical and tracer approaches (Matamala et al. 2003; Mambelli et al. 2011; Martinez et al. 2016). Although RCF appears to be directly tied to photosynthetic activity (Hogberg et al. 2001), the seasonal dynamics of RCF have received limited attention and could influence SOM stabilization. Although soil microbes serve as the principal agents of SOM decomposition, an important regulatory role often is played by invertebrates. Moreover, the soil food web appears to be powered primarily by root-derived inputs (Pollierer et al. 2007). Recent attention to the role of soil macroinvertebrates has been stimulated by observations of invasive earthworms that have been spreading through northern temperate forests, possibly resulting in significant effects on soil C stocks (Bohlen et al. 2004) either by priming SOM decay or stabilizing new soil carbon. The most abundant top predator in these ecosystems, the woodland salamander (*Plethodon cinereus*), can serve as an integrator of C and energy processing in the forest soil (Burton and Likens 1975).

The production and decomposition rate of aboveground plant litter is easily quantified using litter traps and litter bags and by quantifying deposition of woody debris and its decay. In contrast, accurate measurement of root detritus production (i.e. root turnover) is challenging (Tierney and Fahey 2007), and artifacts of confining roots in litterbags may cause error and bias in decomposition studies (Dornbush et al. 2002). The dominant role of root-derived inputs in supplying stabilized SOM, as noted above, is probably explained in part by the proximity of these organic matter sources to sites of stabilization on the surfaces of soil minerals and within soil aggregates (Schmidt et al. 2011). That is, whereas the traditional concept was that resistance to microbial decay was afforded biochemically, more recent work ascribes SOM stabilization

primarily to physical mechanisms that protect inherently labile organic matter from degradative microbial exoenzymes (Dungait et al. 2012). These processes may be sensitive to a variety of environmental and biotic influences including the effects of invasive earthworms (Yavitt et al. 2015). Large, soil-mixing earthworms have invaded extensive areas of northern forests in North America where they were absent prior to European colonization (Frelich et al. 2006). In the absence of earthworms, cold, acidic forest soils accumulate thick organic horizons on the surface, and invasive earthworms can rapidly eliminate these horizons (Alban and Berry 1994), mixing the organic matter into underlying mineral soil. Although this earthworm mixing of surface detritus into mineral soil can promote stabilization of organic matter (Bossuyt et al. 2004), the net effect on soil carbon stocks remains uncertain (Bohlen et al. 2003; Wironen and Moore 2006), and probably depends upon earthworm assemblages and soil properties.

The objective of this contribution is to describe an approach for tracing carbon through forest ecosystems using highly-enriched ^{13}C tracer label *in situ*, in a field setting. We labeled saplings of sugar maple (*Acer saccharum* Marsh) in the understory of a northern hardwood forest by injecting 40 atom %-enriched $^{13}CO_2$ into large sealed chambers (Fig. 1). We conducted a suite of five tracer studies using this label, thereby maximizing the discovery from the expensive labeling process (Figure 2). Our application of these approaches revealed several methodological insights that we hope will be useful to others applying similar methods. This paper details these methodological aspects. Moreover, although some of the results of this research have been published elsewhere (references cited within), the present paper provides an overall synthesis of the project and reports on some additional studies of the fates of fine root and woody detritus, not previously published. We evaluate several key questions concerning forest carbon dynamics: 1) What are the seasonal patterns of C transport through trees and into soil? 2) What is the comparative role of leaf litter vs. roots in supplying soil food webs and SOM pools? 3) How do invasive earthworms influence stabilization vs priming of SOM in aggregates and the movement of C through the soil food web? and 4) What is the contribution of fine root and woody detritus to the formation of SOM in aggregates?

Methods

Study Sites. This research was conducted in two study sites in central New York, the Arnot Teaching and Research Forest (Arnot) and the Turkey Hill Experimental Plantations

(THP). The climate is temperate continental with mean January temperatures of -4°C and in July 22°C. Mean annual precipitation averages 100 cm, evenly distributed year-round. Arnot (42°15'N, 76°40'W) is second-growth Allegheny northern hardwood forest dominated by sugar maple. Soils are acidic Dystrochrepts (pH 4.5-5.0) derived from shallow glacial till overlying upper Devonian shales. Soils are stony, clay content of the <2 mm fraction averages 26% and sand content averages 15% in 0-10 cm depth. In the absence of invasive earthworms, an organic horizon (forest floor) of 3-4 cm thickness is present, but European earthworms have colonized extensive areas (Bohlen et al, 2004), where they eliminate the forest floor horizons through mixing surface detritus and SOM into into mineral soil, as detailed by Fahey et al. (2013a). Although this process can promote stabilization of organic matter (Bossuyt et al. 2004), the net effect on soil carbon stocks is uncertain. The Arnot site characteristics and history are described in detail by Fain et al. (1994).

The THP (42° 27'N, 76° 25'W) are a suite of monospecific, 0.4 ha plots established between 1939 and 1941 by R.F. Chandler of Cornell University to study the effects of trees on soil properties. Trees were planted on abandoned agricultural fields. The research described here was conducted in a pure sugar maple plantation. Soils are coarse-loamy, mixed mesic Typic Fragiudepts developed on silt-enriched glacial till derived from local siltstone. Although earthworms occur in the THP, few earthworms occurred in the study plantation and a thin (2-3 cm) forest floor horizon was observed. Additional detail on the THP is available in Phillips and Fahey (2008).

Isotope Labeling. The forest site at Arnot chosen for ^{13}C labeling was dominated by sugar maple and had been heavily thinned in 2000, releasing abundant advance regeneration of sugar maple. At the time of labeling (2006) the overstory canopy was partially open (Fig. 1), varying from 25-40% canopy closure across the site. Maple sapling density averaged about five stems/m^2 and saplings ranged in height from 0.5 to 2.5 m and diameter at base from 1 to 4 cm. Eight 2.5 m tall by 3 m diameter aluminum frames were positioned around groups of saplings in June 2006 (Fig. 1). The soil and root systems in the chambers were then isolated by trenching to 0.5 m depth, lining the trenches with heavy polyethylene and back-filling with soil. The sides of the chambers were enclosed in 6 mil, greenhouse grade polyethylene sheeting (Dura-Film Super 4), and a removable top was constructed of the same material. Two 10 cm diameter PVC ports were

155 fitted to the chambers to scrub ambient CO_2.

156 Prior to injection of the $^{13}CO_2$ label, the chambers were sealed at dawn and ambient CO_2
157 was scrubbed from chamber atmosphere using a custom-built, closed-loop CO_2 scrub system
158 with 3.5 kg of soda lime. The scrubbing reduced the CO_2 concentration in each chamber to 50-60
159 ppm in 15-20 minutes. Labeled $^{13}CO_2$ (40 atom % enriched; Spectra Stable Isotopes, Columbus,
160 MD) was injected into the chambers until the total CO_2 concentration reached; 500 ppm; this
161 level was chosen so that chamber concentration during drawdown would span the typical value
162 observed in the forest understory (400 ppm). Chamber CO_2 was monitored intermittently using a
163 Li-6200 infrared gas analyzer (LiCor Inc., Lincoln, NE). The air in the chambers was stirred with
164 rotary fans and shade cloth covered the top of the chamber to reduce heat loading (no
165 refrigeration system was used). Labeling was conducted on thirteen sunny mornings between 1
166 and 20 September 2006. Chamber tops were removed when tree uptake had reduced CO_2
167 concentration to nearly constant values, usually about 300 ppm, after 0.5 to 1 hour; hence, a
168 considerable amount of the $^{13}CO_2$ label was lost to the atmosphere. Coarse mesh plastic netting
169 covered the chambers during the ensuing litterfall period to exclude litter from trees outside the
170 chambers. The chamber soils were also treated with ^{15}N labeled fertilizer (see Fahey et al.
171 2013a), but the present paper deals only with C.
172
173 *Study 1.* Primary funding for this research supported studies tracing leaf litter C into soil
174 pools in the presence and absence of invasive earthworms (Fahey et al. 2013b). All leaf litter was
175 collected from the chambers every few days during the litterfall period in October 2006 and
176 2007. Litter was air dried in cardboard boxes until application to field plots in November 2007.
177 We anticipated that the ^{13}C label would be distributed preferentially in non-structural leaf pools
178 in first-year litter and structural pools in second-year litter; this assumption was verified using a
179 sequential extraction procedure (McLeod et al. 2007) and isotopic analysis of the extracts is
180 detailed by Fahey et al. (2011). This procedure yielded estimates of ^{13}C enrichment of free
181 sugars, polysaccharides and pectin, hemicelluloses, and lignocellulose.
182 The leaf litter tracing study was conducted at Arnot in nine sugar maple-dominated study
183 plots with contrasting earthworm assemblages (Table 1); these plots were located about one km
184 from the labeling plots in unmanaged, second-growth sugar maple forest (Fain et al. 1994). One
185 set of three plots was dominated by the anecic (deep burrowing) earthworm *Lumbricus terrestris,*

another set by the epigeic *L. rubellus,* while a third set had no earthworms (control). In each plot, eight 1.0 m^2 quadrats were established and after removing 2007 litter, about 400 g of isotope-labeled leaf litter was added to each quadrat in November 2007 to mimic litterfall in these forests (Fahey et al. 2013c). Half of the plots received the non-structural (first-year) labeled litter and half the structural litter. Plastic edging surrounded each plot, a nylon screen was positioned both below and above to confine the added litter, and a cover board was placed adjacent to each plot to improve salamander capture (Fig. 3).

Calculation of isotope pools and fluxes requires accurate and precise measurements of reference natural abundance as well as soil mass and bulk density. Soil pit excavation (Rowell 1994) was used to quantify bulk density and coarse fragment content and to obtain samples for isotope natural abundance at 5 cm depth interval to 20 cm in each of the nine plots. Four pits were excavated in each plot within 5 m distance from the labeled litter quadrats. Earthworms were extracted in each plot using the "hot mustard" method (Lawrence and Bowers 2002) on three or four 0.25 m^2 quadrats positioned within 3 m of the litter addition quadrats in May 2008, October 2008 and October 2009. Earthworms were returned to the laboratory, sorted by species and processed to determine ash-free dry weight.

Two randomly chosen quadrats were destructively sampled in May 2008 and in October 2008, 2009, and 2010. On each date, the litter remaining between the two screens (i.e. the original applied litter) was collected, the underlying 2 cm soil layer was excavated with hand spades from the entire quadrat and underlying soil was collected by coring to 20 cm depth at several (6-8) locations with a 5 cm diameter soil core. Cores were divided into 5 cm depth increments and pooled for each quadrat. Earthworms for isotope analysis were extracted from the quadrats using the hot mustard method, and salamanders were collected from beneath the cover boards. These samples were returned to the laboratory and processed for isotope analysis; earthworms were dissected and cleaned of gut contents before grinding and whole salamanders were ground for analysis (Fahey et al. 2011).

The initial pool of ^{13}C in each soil layer of each plot was calculated from the mean C concentration, soil mass, and isotope natural abundance from the reference soil pits. The isotope pools at the time of collection were calculated from isotope enrichment assuming bulk density and fine fraction mass were equivalent to plot-wide values. We estimated the % recovery of excess ^{13}C released from the added litter:

$$\%recovery = \frac{(final\ soil\ isotope\ pool - initial\ soil\ pool)}{(final\ litter\ isotope\ pool - initial\ litter\ pool)} x100$$

where, soil or litter isotope pool equals soil or litter isotope atom % ^{13}C * [C concentration] * soil mass.

Study 2 & 3. These studies traced the pulse of photosynthetically fixed ^{13}C label through the trees and into soil pools within the labeling chambers (Fig. 2). For study 2, tissue samples were collected from the trees in the chambers and from similar saplings located within 3 m of the chambers near the end of the labeling period, and then periodically during fall senescence and leaf expansion the following spring (2007). Sun leaves, twigs, and bole wood were collected, and roots were hand-sorted from soil cores. Soil adhering to fine roots was separated by gentle shaking to obtain rhizosphere soil (Phillips and Fahey 2006). Fine roots were collected using in-growth cores. Three 5 cm cores were extracted to 10 cm depth in each chamber in May and July 2007, and root-free soil was added to each core in a mesh bag. After two months cores were extracted and newly-grown fine roots were sorted from soil for isotope analysis. Finally, soil CO_2 emissions were collected for isotope analysis on four dates from the chambers and reference sites in Fall 2006 and Spring 2007 using a NaOH trap. We point out that an unknown proportion of soil C emissions was derived from decay of unlabeled residual roots following trenching in spring 2006. Additional details about these approaches are presented in Horowitz et al. (2009).

For study 3 we traced the longer-term fate of the ^{13}C pulse label. For these studies in the chambers all litter was removed each fall in 2006-2008 and replaced with a roughly equal amount of unlabeled leaf litter. Thus, nearly all the ^{13}C label was delivered to the soil through the tree roots. Soil CO_2 emission was measured within the chambers using a Li-Cor soil respiration chamber and Li-6200 infrared gas analyzer (Norman 1992). Four soil collars were installed in each chamber, and measurements were conducted monthly from October 2006 to 2009, excluding winter months (November-April). To quantify ^{13}C enrichment, the CO_2 emitted from the soil was trapped in NaOH over an 8 h daytime incubation period on the same dates. Following a procedure detailed by Fahey et al. (2013d), ^{13}C of soil emissions was measured on a Finnegan isotope ratio mass spectrometer. Fine roots for isotope analysis were collected by soil coring in August 2007, June and October 2008, and October 2009. For the October 2008 soil

247 cores, microbial biomass C and ^{13}C were measured using a chloroform fumigation-incubation
248 method (Jenkinson and Powlson 1976; Groffman et al. 2015). Also, these 0-5 cm soils were
249 processed for analysis of ^{13}C enrichment of aggregate fractions following a procedure modified
250 from Fonte et al. (2007) and explained in more detail below.
251
252 Soil invertebrates were sampled from the chambers by collecting surface soil horizons on
253 four dates in 2008 and 2009. These samples were returned to the laboratory for invertebrate
254 extractions using Berlese funnels, and reference natural abundance samples were collected
255 within two meters of the chambers. Invertebrates were identified to order or family by the
256 Cornell Insect Diagnostic Laboratory. We also extracted earthworms from chamber soil for
257 isotope analysis in fall 2007 and spring 2008 and 2009. Samples were cleaned of gut contents
258 before analysis. Finally, red-backed salamanders were collected beneath cover boards in the
259 chambers in fall 2008 and summer 2009 and analyzed for isotopes.
260
261 *Study 4 & 5.* These studies took advantage of the highly enriched tree tissues to quantify
262 long-term decay and incorporation of detrital C into soil aggregate pools (Fig. 2). Highly
263 enriched fine roots were collected from the labeling chambers when isotopic enrichment of fine
264 roots reached peak values (Fahey et al. 2013d). Roots were air-dried and subsamples were taken
265 for C and ^{13}C measurements. Root samples were added to sieved soil obtained from the forest.
266 Each core received about 0.6 g of fine roots to roughly match fine root density in these forests
267 (Fisk et al. 2004). The root-soil mixtures were incubated in 5cm diameter PVC cores with holes
268 drilled in the sides to allow ingrowth of roots and fungal hyphae. The cores were installed at the
269 THP sugar maple plantation in November 2012. Several control soil cores without ^{13}C roots
270 added were included for periodic reference collections. Randomly chosen sets of these root
271 decay cores were collected in November 2013, 2014, 2015 and 2018 (1, 2, 3 and 6 yr of
272 incubation). Cores were returned to the laboratory and processed for isotope analysis of bulk soil
273 and aggregate fractions using the approach below.
274 Highly enriched sugar maple stem tissue for studying wood decay was collected from the
275 chambers by harvesting the trees. The stems were air-dried and cut into numerous 20 cm
276 sections, 2 - 4 cm diameter. Stem sections were bundled into 60 samples averaging about 1200 g
277 air-dry weight. Subsamples were obtained to measure moisture content and dried to constant

mass; average sample oven-dry weight was 887 g. Wood decay samples were enclosed in coarse-mesh nylon screens and placed in contact with the soil surface in two pure sugar maple stands: 1) near the labeling chambers at Arnot and 2) the same maple plantation at THP where root core samples were incubated. Eighteen samples distributed across sites and treatments (i.e. 2 sites x 9 replicates) were collected after 3 years and 6 years. After removing the bundles, the top 1 cm of soil underlying each bundle was collected by scraping with a garden spade. A 5 cm diameter soil core was collected to 5 cm depth beneath each bundle. In the laboratory each bundle was carefully cleaned of adhering debris and the remaining stem tissue was dried, weighed, and ground for isotope analysis. The scrape and core soil samples were processed for isotope analysis after aggregate separations, described below.

Soil aggregates. Soil samples from all these studies were fractionated for water-stable aggregates by wet-sieving of air-dried subsamples of the <2 mm fraction following a modification of the procedure of Elliott (1986), as detailed by Yavitt et al. (2015). Briefly, soil samples were slaked in tap water on a 250 μm sieve for 5 minutes and then the sieve was gently shaken at 25 strokes/min for 2 minutes. Floating material was decanted and is designated light POM. Material that passed through the sieve was either collected on or passed through a 53 μm sieve; hence, three further fractions were collected: the <53 μm fraction (silt plus clay), 53-250 μm fraction (microaggregates), and >250 μm fraction (macroaggregates). The fractions were dried to constant mass at 60°C and weighed. A subsample of the macroaggregate fraction was further separated following Bossuyt et al. (2004). Macroaggregates were shaken with stainless steel ball bearings while submerged in water on a 250 μm sieve to break apart aggregates. A continuous stream of water flushed particles through the sieve to avoid breaking apart microaggregates, and the <250 μm fraction was further sieved through 53 μm sieve to separate silt and clay from microaggregates held within the macroaggregates. Each fraction was oven dried and weighed. Finely ground subsamples of the sieved aggregate fractions were analyzed for ^{13}C.

Isotope concentrations in soil and tissue samples were determined on a Finnegan Isotope Ratio Mass Spectrometer at the Cornell Stable Isotope Laboratory, with appropriate standards for normalization correction, instrument linearity, and precision purposes. It was important to keep separate the samples for natural abundance ^{13}C from the highly enriched, labeled samples and to avoid carryover in the analysis between samples with highly contrasting enrichment.

Descriptions of the statistical approaches employed in this suite of studies are available in other publications (Fahey et al. 2013a,b,d); for reasons of length, we do not repeat the details here. In general, for the chamber studies we used ANOVA with chambers as replicates, and in the forest plots a mixed model with plot as random effect and worm treatment, soil depth, collection date and slope position as fixed effects. We simply refer to differences that were statistically significant ($p < 0.05$) in this paper.

Results

Natural abundance of ^{13}C in soils. We observed significant variation in ^{13}C natural abundance among the study plots at Arnot. Average $\Delta^{13}C$ in no-earthworm plots ranged from -25.03 to -25.89 across all depths and slope positions. Natural abundance of ^{13}C increased (i.e. less negative) at greater depths in soil and was significantly higher in lower slope positions. Natural abundance of ^{13}C throughout the 0-20 cm soil was similar in no earthworm plots and in plots dominated by *Lumbricus rubellus* but significantly lower in those dominated by *L. terrestris.* The ^{13}C natural abundance pool in soils also varied significantly across the landscape, mostly reflecting differences in $\Delta\ ^{13}C$.

Isotope Labeling. The ^{13}C labeling procedure was successful at strongly enriching two year-classes of foliage. Average enrichment of ^{13}C in the second-year leaf litter (418 per mil, "structural" label) was much greater than for the first-year litter (142 per mil; "non-structural" label), illustrating the high capacity of these saplings to store assimilated C overwinter for allocation to shoots the following spring. Among the seven chambers in which labeling was conducted we observed roughly two-fold variation in ^{13}C enrichment.

As expected, the distribution of the ^{13}C label differed between the structural (2nd year) and non-structural (1st year) leaves. Most notably, the label concentration was greater in the lignocellulose fraction in the former and lower in this fraction in the latter. Conversely, the hemicellulose and free sugar fractions were preferentially labeled in the non-structural and weakly labeled in the structural litter. Peak enrichment of the fine root pool was observed in April 2007, eight months after labeling and was considerably lower than for leaf litter (106 per mil).

Tracing the ^{13}C label in the chambers. We labeled the trees in late summer hoping that

much of the label would be retained in leaf litter. As expected, ^{13}C enrichment of foliage declined considerably in the month following labeling, declining from 387 (±14) per mil to 174 (±17) in late stages of senescence. This decline represented both respiratory losses as well as foliar resorption; the significant role of the latter was emphasized by coincident increases of ^{13}C enrichment of recent stem wood during the same time period (from 152 per mil to 500 per mil). Very high enrichment of soil CO_2 emissions at this time ($\Delta^{13}C$ = 766 per mil) indicated the importance of late-season photosynthesis in supplying root metabolism. In contrast, new fine root growth in spring, measured with ingrowth cores, was only weakly enriched (-17.8 per mil) whereas new twigs (1,056 per mil) and swelling buds (1,335 per mil) were highly enriched, illustrating the unidirectional transport of stored assimilates. Enrichment of ^{13}C in fine roots in fall (89 per mil) was much greater than for rhizosphere soil (-23.0 per mil)

In the longer term, ^{13}C enrichment of soil CO_2 emission in the chambers declined from peak values in fall 2006 to intermediate values during the growing season in 2007 (average=64.2 per mil) and low values in 2008 and 2009 (-17.7 per mil). The rhizosphere soil pool increased gradually from fall 2006 (-23 per mil) to spring 2007 (-13 per mil) and reached a peak value in late summer 2007 (-2.0 per mil), before declining thereafter. Similarly, ^{13}C enrichment of newly grown fine roots increased from early summer 2007 (-17.8 per mil) to late summer 2007 (-16.9 per mil), peaking in spring 2008 (-13.6 per mil) before declining to natural abundance during summer 2008 (-27.8 per mil).

Whole-ecosystem budget estimates were calculated using the measurements of ^{13}C pools and fluxes in the chambers during the two years following labeling. Total belowground carbon allocation (TBCA) of the ^{13}C pulse was estimated at 3,207 mg $^{13}C/m^2$, the sum of $^{13}CO_2$ flux via soil respiration (2,340 mg excess $^{13}C/m^2$) plus the maximum measured pool size in roots and soil (867±215 mg $^{13}C/m^2$). The immediate allocation of this TBCA to root and rhizosphere soil respiration was about 40%, based on measurements of fall/winter soil respiration in 2006-2007. During the first dormant season rhizosphere carbon flux was estimated at 7.5% of TBCA and allocation to new root growth in the year following labeling at a minimum of 9% of TBCA (assuming no turnover of new roots collected using in-growth cores). By comparison, the pool of excess ^{13}C in microbial biomass in 0-10 cm soil in the chambers was very small (3.9 mg $^{13}C/m^2$) even though this pool was highly enriched (Δ ^{13}C=1±3‰).

Enrichment of ^{13}C was measured for two years following labeling (2007-2008) in litter

371 invertebrates, earthworms and salamanders. Highest enrichment was observed for tissues of
372 lumbricid (saprophagous) earthworms, with values peaking in June 2008 at values comparable
373 to fine roots (Fig. 4); significantly lower enrichment was noted for geophagous earthworms. The
374 principal microarthropods, Acari and Collembola, showed enrichment similar to the microbial
375 biomass. Surprisingly, predatory arthropods (Arana, Diplopoda, Coleoptera) had similar peak
376 enrichment as their presumed prey the microarthropods. Moreover, very high enrichment was
377 observed for ants (Formicidae), dominated by *Lasius* spp,,and adult weevils, mostly *Barypeithes*
378 *pellucidus*. The excess ^{13}C pools in the litter arthropods (based on del^{13}C and measured biomass)
379 were much lower (0.05-0.09 mg $^{13}C/m^2$) than for microbial biomass. Finally, tissues of the top
380 predator the woodland salamander, *Plethodon cinereus*, were highly enriched in the chambers by
381 October 2008 ($\Delta^{13}C$=25.9$\pm$1.8‰ vs natural abundance of -23 ‰), though much less so than
382 their principal prey (Fig. 4). Surprisingly, these values greatly exceeded the modest enrichment
383 of salamander tissues in the litter decay plots (see below).
384
385
386 *Tracing ^{13}C labeled leaf litter.* In the absence of earthworms, decay of ^{13}C-labeled leaf
387 litter applied to plots at Arnot Forest followed an exponential pattern based on dry weight loss
388 and ^{13}C pool changes. Although the decay rate of the non-structural labeled litter was
389 significantly higher during the first six months (November-May) this difference was not
390 observed thereafter over three years of decay. After three years 38% of the initial added ^{13}C label
391 was recovered in this litter pool (Fig. 5). As expected, earthworms greatly accelerated the
392 disappearance of ^{13}C from the added litter, with only 17% remaining after one year and virtually
393 complete disappearance after two years (Fig. 5). Note that in Figure 5 the loss of C from the plots
394 has been calculated by difference based on the mass balance of C addition and C recovery in soil
395 pools (e.g., soil ^{13}C emissions were not directly measured).
396 In the absence of earthworms we detected ^{13}C enrichment of forest floor and mineral soil
397 to a depth of 10-15 cm in May 2008, six months after litter addition (Fig. 6); significant excess
398 ^{13}C was not detected at 15-20 cm depth. We calculated recovery of ^{13}C in soil as a percentage of
399 measured ^{13}C release from litter for each individual sample plot. After six months (May), an
400 average of 39.5% of the ^{13}C released from labeled litter was recovered with the highest
401 proportion in 0-5 cm mineral soil. Among the soil aggregate fractions, the highest enrichment

was observed in silt and clay, reflecting adsorption of dissolved organic C leached from the decaying litter. By October 2008 the excess ^{13}C recovered in soil declined significantly and none was detectable below 10 cm depth. No difference in % recovery was observed between the non-structural and structural labeled litter.

Percent recovery of label in soil microbial biomass averaged 2.3% of that released from litter in May with roughly equal amounts recovered in forest floor and 0-10 cm mineral soil and none detected below 10 cm. Higher % recovery was observed for the non-structural than the structural labeled litter. Conversely, fungal hyphae collected using soil bags in upper mineral soil were more highly labeled for the structural litter (9 vs 3 ng ^{13}C/g soil).

Earthworms greatly altered these patterns of label recovery in soil (Fig. 7). In particular, ^{13}C was recovered to 20 cm depth and the percent recovery was much greater, as high as 80% in May 2008. However, just as in no earthworm plots ^{13}C recovery declined markedly during the first summer, probably primarily reflecting higher soil temperature and microbial metabolism (but see General Discussion). After two years of decay an average of 22% of the ^{13}C released from litter was recovered in soil in the earthworm-invaded plots (Fig. 5). Although the patterns of soil ^{13}C enrichment were qualitatively similar in the *L. terrestris*-dominated plots and the *L. rubellus* plots, incorporation of the label was faster and to a greater depth in the former (Fig. 7). Recovery of ^{13}C in microbial biomass was similar between the no-earthworm and invaded plots; it increased during summer 2008 to over 3% before declining to less than 1% in year 2 (October 2009). Earthworm tissue was highly enriched in ^{13}C and represented 0.4 to 0.6% recovery of ^{13}C released from litter.

Recovery of ^{13}C among soil aggregate fractions was strikingly different between no-earthworm and invaded plots; earthworms greatly promoted the incorporation of litter ^{13}C into soil aggregates. For example, after six months macroaggregates and free microaggregates were the most highly enriched fractions in the presence of earthworms. After two years this pattern was reinforced and microaggregates occluded within macroaggregates were the most enriched fractions (Fig. 8); the silt plus clay fraction was less enriched. Not surprisingly, the light POM fraction, presumably mixed into mineral soil by earthworms, comprised a significant proportion of ^{13}C recovery.

By the first spring (May 2008), tissue concentrations of ^{13}C were measurably enriched in terrestrial salamanders (*P. cinereus*) from plots with and without earthworms (Fig. 9). For

433 earthworm invaded plots, the increased label in salamander tissues from plots receiving 2nd year
434 leaves compared to plots receiving 1st year leaves was generally proportional to differences in
435 tracer concentration between the two litter types (i.e. higher in 2nd year leaves); however, in the
436 absence of earthworms, tracer concentrations in salamanders from plots receiving 2nd year leaves
437 was proportionally much higher than the differences in leaf concentrations. Therefore, in the
438 absence of earthworms, salamanders accumulated a greater proportion of C from structural than
439 labile components of leaves.
440 Mean salamander tracer concentrations in worm plots peaked earlier than in no worm
441 plots and peak enrichment in salamanders was much higher in no worm than earthworm invaded
442 plots (Fig. 9). Moreover, peaks were later in plots with ^{13}C labeled structural leaf tissues (data
443 not shown). Salamander tracer concentrations had returned to reference baseline levels after 3
444 years on plots with labile leaf tracer only. In contrast, salamander tracer concentrations on plots
445 with ^{13}C labeled structural leaf tissues were still measurably enriched at ~20% of peak
446 concentrations. Integrated over three years and compared to plots without invasive earthworms,
447 earthworms reduced mean ^{13}C tracer in salamanders by 73% - 78% on plots that received ^{13}C
448 labeled structural leaf tissues. On plots that received leaves with tracer primarily in labile forms,
449 earthworms reduced ^{13}C tracer in salamanders by 45% on plots dominated by *L. terrestris* but
450 only 1% on plots dominated by *L. rubellus*.
451 *Carbon flow from fine root and woody detritus into soil aggregate pools.* We quantified
452 the mid-term (6-year) incorporation of ^{13}C in fine root and woody detritus into SOM, and we
453 calculated total recovery as the sum of excess ^{13}C in various aggregate fractions. Fine roots
454 dispersed in soil at densities comparable to our forest soils were mineralized very rapidly, with
455 only 18.7 +/- 1.4% remaining after two years. After six years an average of 10.8±1.0% of the
456 original label in fine roots was recovered in SOM. Throughout the course of fine root
457 decomposition over half of the label was recovered in the low-density POM fraction (Fig. 10a).
458 This proportion did not change systematically over time. Carbon concentration in this light POM
459 fraction declined through time from an average of 39.8% at time zero to 36.3% after one year
460 and leveling off thereafter at 23.6% C. Recovery of the label in macroaggregates declined
461 steadily through time from 4.7% at year 1 to 2.0% by year 6 whereas recovery in
462 microaggregates (2.0%) and silt and clay fractions (0.8%) remained roughly constant through
463 time.

464 Decomposition of small stems (2-4 cm diameter) lying on the soil surface was rapid;
465 based on collections at three and six years the exponential decay coefficient (k) at the THP site
466 was -0.545 yr^{-1}, and after six years only 3.8 $\pm$0.7% of the wood mass was recovered in the decay
467 bags. Wood decay at the Arnot site was significantly slower, with an exponential decay
468 coefficient (k) value of -0.28 yr^{-1} and 18.6 $\pm$ 2.8% remaining after six years. In the former site
469 we observed saproxylic soil invertebrates (Scarabaeidae) that were not present at the latter site.
470 After six years at THP, where little wood remained, we recovered only 0.33$\pm$0.03% of the ^{13}C
471 added in the wood samples in soil to 5 cm depth. Of this recovery in SOM, 42% was in the
472 surface 1 cm of soil and 58% in underlying mineral soil. In contrast, only 0.14 +/- 0.006% was
473 recovered in the Arnot soil where decay was slower. Among the aggregate fractions, ^{13}C
474 recovery was highest for macroaggregates with moderate amounts in microaggregates and light
475 POM and small amounts in silt and clay fraction (Fig. 10b). Five times higher recovery in the
476 light POM fraction was observed at THP as at Arnot.
477
478 **Discussion**
479 We employed a stable isotope tracer approach to evaluate the flow of carbon through
480 sugar maple forest from the trees (saplings) to detritus, through the soil food web into soil
481 aggregate fractions, and we quantified the role of invasive earthworms on these processes (Fig.
482 2). We begin with detailed discussion of the methods employed and the lessons learned to help
483 guide future efforts using these approaches. We follow with a general discussion of the
484 implications of our studies of forest ecosystem carbon flow for understanding the dynamics of
485 these key processes in the global carbon cycle.
486
487 *Isotope Labeling.* We required a large quantity of highly enriched leaf litter labeled with
488 ^{13}C to be able to detect the label in soil. We used a suite of eight independent 3 m diameter
489 chambers containing 10-year-old understory saplings and enclosed in plastic with removable
490 tops (Fig. 1). In retrospect, it might have been feasible, even in this remote forest setting, to
491 construct a recirculating system connecting the chambers and thereby to achieve a greater
492 proportional recovery of the added label; however, the distance between our chambers (up to 70
493 m) made the plumbing of such a system impractical.
494 Several features of our procedure proved to be crucial to success. Labeling saplings early

in the morning in the understory of a heavily thinned forest allowed rapid label assimilation and avoided heat damage; thus, following scrubbing of the chamber atmosphere and injection of the label, the saplings were able to draw down chamber CO_2 from 500-550 ppm to roughly steady-state values (ca. 250-300 ppm) within one hour and then chamber tops were removed to dissipate heat. Notably, for one chamber in a more shaded location, minimal drawdown was achieved and the chamber was abandoned; thus, a fine balance exists between sufficient light to power photosynthesis but avoiding heat stress. We labeled at the end of summer so that respiratory losses of $^{13}CO_2$ prior to leaf abscission would be minimized and leaf litter maximally labeled. In total, about 30 kg of isotope labeled leaf litter was produced.

First-year leaf litter had much lower ^{13}C enrichment ($\Delta^{13}C$=142‰) than second-year litter ($\Delta^{13}C$=418‰) illustrating the high capacity for overwinter storage of photoassimilates. We also showed that labeling of biochemical pools differed between these cohorts, the first-year litter favoring more labile pools and the second year the structural, lignocellulose. Although both cohorts were air dried in the same facility, the longer storage time of the first cohort may have altered decomposition (Niklinska et al. 1998), contributing to its significantly slower decay during the first six months (Fahey et al. 2011). For a more reliable comparison between litter types, better control of artifacts due to storage is needed.

Chasing the ^{13}C pulse label in the chambers. Some significant challenges prevented precise quantification of the movement of the pulse label within the chambers. Perhaps most importantly, we did not quantify gross primary production or net assimilation of the ^{13}C pulse. Because we labeled all the chambers simultaneously, careful measurement of $^{13}CO_2$ concentration at the peak and minimum during each labeling interval was not possible with the personnel and equipment available. We also emphasize that monitoring CO_2 with an IRGA required correction for the differing IR absorption by $^{12}CO_2$ vs $^{13}CO_2$ (Svejcar et al. 1990). For future work employing this approach to trace photoassimilate supplied through the root channel, we recommend deploying sufficient personnel and equipment to carefully monitor chamber $^{13}CO_2$ through the entire labeling period, and to quantify the C budget within the chambers (i.e GPP, NPP, NEP).

Although nearly all the soil excess ^{13}C in the chambers was derived from the root channel, an unknown (but probably small) amount was associated with canopy leaching and

526 leaching from fresh litter by rain events that occurred during the senescence period. An
527 additional source of error resulted from severing the roots of trees surrounding the chambers;
528 thus, although excess $^{13}CO_2$ flux from soil respiration was associated primarily with
529 heterotrophic and autotrophic processing of the added ^{13}C, an unknown amount of the total CO_2
530 emission was derived from decay of residual non-labelled roots. This source of uncertainty could
531 be reduced by delaying labeling for a year or two after chamber installation to allow decay of the
532 severed roots.
533 *Tracing leaf litter ^{13}C into soils and the soil food web*. Because of the high enrichment in
534 litter, we were able to detect even the relatively small proportion that accumulated in the soil
535 (<3%; Fig. 4). We emphasize that we would have been unable to conclusively detect this soil C
536 if we had measured only site-wide or depth-averaged values of natural abundance ^{13}C. Thus, it is
537 important to obtain detailed measurements of soil $\Delta^{13}C$ in the immediate vicinity of litter
538 application plots and to achieve very high enrichment of the applied detritus (see "Tracing fine
539 root and wood detritus", below) in order to quantitatively trace ^{13}C in soils and food webs.
540 Another procedure that proved effective was applying labeled litter to large quadrats (1.0
541 $m^{2;}$ Fig. 3) and at the time of sampling to first scrape the top 2 cm of surface soil underlying the
542 entire plot and to composite several 5 cm diameter soil cores to obtain deeper soil (here, to 20
543 cm). This approach overcame small-scale spatial variation in label distribution resulting from
544 favored hydrologic flow paths. It proved effective for demonstrating the adsorption of litter-
545 derived DOC in mineral soil during the first six months, as well as subsequent mineralization of
546 most of that ^{13}C during the following warm season (Fig. 4; Fahey et al. 2011).
547 We extracted earthworms from both the litter addition plots and the labeling chambers and
548 measured tissue concentrations and pools of ^{13}C by dissecting and cleaning their gut contents
549 before isotope analysis. We sorted these earthworms by species which proved informative as
550 much higher ^{13}C levels were observed in litter feeding *Lumbricus* spp than in endogeic
551 earthworms (e.g., *Octolasion tyrtaeum*) in the litter addition plots (Fig. 4); surprisingly, the same
552 pattern, though less pronounced, was observed for earthworms collected from the labeling
553 chambers (Gilbert et al. 2014), indicating that *Lumbricus* spp. feed on fine roots and their decay
554 products. Recovery of litter-applied ^{13}C in earthworms in the field plots averaged about 0.5%
555 across plots and years (Fahey et al. 2013a).
556 We were able to detect the ^{13}C label in microbial biomass using the chloroform

557 fumigation-incubation method (Jenkinson and Powlson 1976; Groffman et al. 2015), but only in
558 the upper 10 cm of soil. As with the earthworms, percent recovery was higher for the non-
559 structural labeled litter; presumably this difference reflects higher bacterial utilization of the non-
560 structural label because chloroform fumigation-incubation preferentially measures bacterial
561 biomass (Harris et al. 1997). Conversely, greater recovery of ^{13}C in fungi, collected using
562 ingrowth bags, was observed for the structural litter, suggesting that fungi more efficiently utilize
563 lignocellulose components.
564 The inclusion of a cover board in the center of each litter addition plot (Fig. 3) facilitated
565 collection of the principal top predator in the soil food chain, the Eastern red-backed salamander
566 (*Plethodon cinereus*). This approach proved successful as 340 salamanders were collected from
567 the 72 plots over the course of the study. These salamanders have very small home ranges
568 (generally < 1 m^2; Mathis 1991), and we presume they fed frequently in the labeled litter, which
569 is supported by evidence that the tracer was measurable in salamanders within 6 months (Fig. 9).
570 The timing of label enrichment in salamander tissues peaked earlier in the earthworm-invaded
571 plots, reflecting earthworm acceleration of C transformation and salamander consumption of
572 earthworms. However, we demonstrated that ultimately the more rapid redistribution of tracer
573 into soil microbial pools and reduced abundance of soil invertebrates caused a net reduction in
574 leaf litter carbon flow to salamander populations (Fig. 9).
575
576 *Carbon flow from fine root and woody detritus to SOM pools*. We achieved very high
577 enrichment of fine roots as well as stem wood which allowed us to detect even the relatively
578 small amounts of label added to soil aggregate pools from these detrital sources. Our aggregate
579 separation procedure afforded reliable estimates of total ^{13}C recovery (an indicator of overall fine
580 root decay) and the distribution of the residual fine root detritus among soil aggregate fractions
581 (Fig. 6).
582 Calculations of ^{13}C recovery in soil from the decaying fine roots required reference
583 values for isotope natural abundance of the soils. Variation in ^{13}C natural abundance in the bulk
584 soils was low (CV= 2%); higher variation was observed for aggregate fractions separated from
585 root-free control cores that were collected along with the samples, with CVs ranging from 3% to
586 9% across the six aggregate fractions; the highest variation was in the macroaggregate fraction
587 (CV=6.3 to 8.2%) and lowest for the low-density POM (CV=3%). No systematic temporal

588 differences in natural abundance were observed across the four fine root core collection dates,
589 and we used the mean values from all the control samples to calculate ^{13}C recovery. A few
590 outlier values (over 10% deviation) in control fractions had to be discarded; whether these
591 anomalies were the result of analytical error, contamination or problems with the subsampling or
592 fractionation procedures is unknown.
593 After six years of fine root decay, variation in percent ^{13}C recovery in the cores was
594 remarkably low; for example, the CV for total recovery, across 19 cores, was 39.6% after six
595 years. Not surprisingly, variation in recovery among aggregate fraction was higher; for example,
596 the CV for the largest fraction, light POM, was 54.4% and that for free microaggregates, 116%.
597 Our approach for measuring fine root decomposition avoided possible artifacts of root
598 litter bags for which extremely high concentrations of root material are incubated usually without
599 direct contact with soil. The root densities in our cores (equivalent to 300 g/m^2) were comparable
600 to those in forest soils from our study area (Fisk et al. 2004) so that interaction between the soil
601 substrate and decaying roots was semi-natural. Root litter bag studies have often noted
602 unexpectedly low decay rates (McClaugherty et al. 1984; Silver and Miya 2001), and studies
603 using intact cores have shown much higher rates (Dornbush et al. 2002; Li et al. 2015).
604 Moreover, using an approach similar to ours, Santos et al. (2016) measured fine root decay rates
605 for red maple (*Acer rubrum* L.) very similar to ours. We conclude that decay rates of first to
606 fourth order fine roots of maple are very rapid (k=0.9 yr-1).
607 Our approach for measuring the fate of C in small wood (2-4 cm diameter) was effective
608 because of the high ^{13}C enrichment (1.162 atom % 13C) and the relatively large amount of wood
609 added to each plot (mean= 887 g/plot or 30 kg/m^2). Thus, despite very low recovery of wood-
610 derived ^{13}C after six years of decay (0.33%; i.e. presumably nearly all the wood was mineralized
611 to CO_2) variation in recovery across plots was surprisingly low (CV=32% for n=12 plots).
612 Moreover, variation in the distribution of label recovery across aggregate fractions also was
613 moderate (CV ranging from 28% for macroaggregates to 51% for microaggregates). Recovery
614 across aggregate fractions for the fine root decay cores was considerably higher; for example,
615 after six years of decay the CV values for the wood samples were smaller than for the fine root
616 cores (see above), presumably primarily because of the much higher total amount of label added
617 in the wood compared to the fine roots.
618

General Discussion. Although we were unable to quantify ^{13}C assimilation by the saplings in the chambers, our short-term tracing of the late summer ^{13}C pulse through the trees and soil provided some qualitative support for concepts of carbon partitioning as well as some useful new insights into belowground processes. As elucidated by Ekblad and Hogberg (2001), new photosynthate immediately supplies root and rhizosphere respiration in forest soils. Thus, our late-summer pulse of ^{13}C assimilate immediately (within 6 days) appeared as very high enrichment of both soil CO_2 efflux and root tissues (Fahey et al. 2011). The following spring the strong sink in growing shoots and leaves apparently resulted in largely unidirectional C transport from storage pools in roots and stems; surprisingly, new fine roots collected with ingrowth cores exhibited minimal ^{13}C enrichment. Gaudinski et al. (2009) demonstrated that new root growth in trees is supplied by both stored C and fresh photosynthate; in these sugar maple saplings root growth the following season clearly was not supplied significantly from late-summer stored photosynthate, implying that new photosynthate and older stored C were the principal sources (Horowitz et al. 2009).

Rhizosphere C flux (RCF) is a major pathway of forest C flux and experimental studies indicate that RCF is driven in part by photosynthetic activity (Kuzyakov and Cheng 2001). Phillips and Fahey (2005) used a ^{13}C pulse labeling approach to demonstrate that RCF during the growing season constituted at least 7% of annual photoassimilation in sugar maple saplings. Our observations of increasing ^{13}C enrichment in rhizosphere soil outside the growing season (October-May; Fahey et al. 2013d) clearly indicated that RCF is active in the absence of photosynthesis and may constitute a comparable flux to that during the growing season. Future work on non-growing season RCF in temperate forests is clearly warranted.

The traditional view of biochemical recalcitrance of plant and microbial products has been partly superseded by the concept of SOM stabilization by biophysical inaccessibility associated with soil aggregation and organic matter-mineral complexation (Kleber 2010, Schmidt et al. 2011, Dungait et al. 2012). In the absence of invasive earthworms, we observed that the initial stages of leaf litter decomposition was marked by considerable leaching of DOC that was subsequently adsorbed onto the surfaces of soil minerals as previously shown (Qualls and Haines 1992, Kaiser et al. 1996). About 40% of the ^{13}C released from litter between November and May was recovered in soil (Fig. 4), and our aggregate fractionation indicated that the silt plus clay fraction was most strongly enriched in ^{13}C (Fig. 5). However, in support of the

650 contention of Kaiser and Guggenberger (2003), most of this adsorbed SOM remained labile, as
651 this pool declined dramatically during the following summer (Fig. 4) probably primarily
652 reflecting higher microbial metabolism at higher soil temperatures. The role of microbial activity
653 driving this pattern is emphasized by the high recovery of litter ^{13}C in soil microbial biomass
654 during the first year of decay (Fig. 4). However, an additional process that could account for a
655 significant loss of ^{13}C from surface soil is eluviation of clay and silt by vertical water movement
656 (Thorp et al. 1959); perhaps some of the ^{13}C enrichment at 10-20 cm depth after 1-2 years (Fig.
657 6) is accounted for by this process.
658 Colonization of forest soils by invasive earthworms can cause profound changes in the
659 amount and distribution of soil C (Bohlen et al. 2003) as well as the entire soil food web. In cold
660 temperate forests where organic horizons (forest floor) accumulate in their absence, several
661 processes influence the effects of earthworm invasion on soil C stocks: 1) through their feeding
662 and mixing activity invasive earthworms can eliminate forest floor horizons over decadal time
663 scales, reducing C stocks in surface soil (Alban and Berry 1994); however, 2) this mixing
664 activity also can increase C stocks in underlying mineral soil (Wironen and Moore 2006); 3)
665 earthworm feeding and casting activity can prime microbial utilization of pre-existing SOM
666 (Brown et al. 2000); 4) conversely, by incorporating detrital organic matter into soil aggregates
667 and promoting interaction between detrital organic matter and soil minerals, earthworms
668 contribute to stabilization of SOM through biophysical effects (Bossuyt et al. 2005, Seeber et al.
669 2006); and 5) finally, because recent studies suggest the predominance of root-derived organic
670 matter as the source of forest SOM (Matamala et al. 2003; Mambelli et al. 2011), any alteration
671 of root dynamics by earthworms could profoundly affect soil C stocks. Ultimately, the long-term
672 effects of earthworm colonization of temperate forest soil on soil C stocks depends upon the
673 balance among this complex suite of influences.
674 Our studies provide evidence on this important issue as earthworms continue to colonize
675 forests across north temperate zones (Frelich et al. 2006). In our study area at Arnot Forest
676 earthworm colonization reduced total soil C stock in the upper 15 cm of soil by 27% (Bohlen et
677 al. 2004) as elimination of forest floor and priming of mineral soil organic matter exceeded
678 stabilization effects; however, the time scale over which this change occurred is unknown. Our
679 litter carbon tracing in the same study area provides empirical evidence on the effects of
680 earthworm activity after forest floor horizons have been eliminated by earthworms. Certainly, in

681 the initial stages of decay, the mixing of litter into mineral soil by earthworms contributed much
682 more C to mineral soil horizons than is supplied by leaching and adsorption of DOC in the
683 absence of earthworms (Fig. 4); notably, the earthworm assemblage dominated by *L. terrestris*
684 more rapidly and deeply mixed this leaf litter into the soil (Fig. 6), reflecting the feeding and
685 litter incorporating behavior of this species. However, much of the detrital organic matter
686 supplied by earthworm mixing was rapidly decomposed so that after two years only 25% of the
687 added litter ^{13}C remained in the soil. In contrast, without earthworms over half of the detrital C
688 remained, mostly in the surface organic horizons. During the third year of decay in the worm-
689 invaded soils only a relatively small amount of the ^{13}C label was lost from mineral soil (about
690 12%), suggesting that a considerable proportion of the litter C may be stabilized. The mechanism
691 of stabilization was indicated by the distribution of the label in soil aggregate fractions; highest
692 ^{13}C enrichment was observed in macroaggregates and especially in microaggregates held within
693 them (Fig. 8; Yavitt et al. 2015). Clearly, earthworm activity promoted the incorporation of
694 particulate organic matter into microaggregates where some of it may be physically inaccessible
695 to microbial decay (Six and Paustian 2014). Although short-term studies suggest that earthworm
696 promotion of SOM stabilization can exceed effects on C mineralization (Zhang et al. 2013), it
697 remains uncertain whether this net effect applies over longer time scales. Our results suggest that
698 earthworm effects on C mineralization may exceed effects on stabilizing soil carbon, reinforcing
699 the observations of earthworm-induced declines in soil C in our study area (Bohlen et al. 2004).
700 Future studies analogous to ours need to sample beyond the three-year time scale.
701 The effects of earthworm colonization on forest soil C dynamics is further complicated
702 by their influence on root dynamics; earthworm effects on roots could override or reinforce their
703 effects on aboveground litter. In our study sites at Arnot Forest fine root biomass was about 25%
704 lower in earthworm-invaded than uninvaded soils (Fisk et al. 2004). One mechanism that could
705 contribute to this difference is earthworm consumption of fine roots; our observation that
706 earthworms collected from the chambers within a few weeks of labeling were much more highly
707 enriched in ^{13}C (33 per mil) than rhizosphere soils (-23.0 per mil) indicated that they were
708 consuming live roots (89 per mil) (Horowitz et al. 2009; Gilbert et al. 2014). Moreover,
709 earthworm activity in two of our labeling chambers promoted the incorporation of fine root
710 detritus into soil aggregates in comparison with chambers lacking earthworms (Fig. 4; Yavitt et
711 al. 2015) matching the observations of Sanchez-de Leon et al. (2014). Thus, it is possible that

712 earthworm activity could increase stabilization of fine root detrital C, a topic deserving further
713 detailed study.
714 Our study tracing the products of fine root decomposition indicated that 18.7% of the
715 original ^{13}C was recovered in the soil after two years, a value remarkably similar to that
716 measured for red maple roots (20.7%) using a similar approach (Santos et al. 2016). These
717 authors speculated that low recovery of fine root C in their site was partly a result of the limited
718 SOC complexation associated with their sandy soils; however, we found a similarly low value on
719 a soil with moderately high clay content (26%). About half of the remaining fine root C was
720 mineralized over the next four years, as 10.8 +/- 1.0 % (n=19) of the original ^{13}C was recovered
721 in the soil after six years. Somewhat more than half of this C was recovered as low-density
722 particulate C (6.7 % recovered) with the remainder in aggregates or clay complexes. The fact
723 that C concentration of the light POM fraction declined continuously through time during decay
724 suggests that POM became encrusted with mineral particles soil minerals, perhaps promoting its
725 stability. How this recovery fits with leaf litter "limit values" is somewhat ambiguous; Berg et al.
726 (1996) proposed the "limit value" as the maximum extent of mass loss for plant litter, but how
727 our recovery of fine root C fits with leaf litter limit values is ambiguous because litter decay
728 studies do not measure recovery in soil. By comparison, Lovett et al. (2016) observed a limit
729 value for sugar maple leaf litter of about 80%, lower than for fine roots (89.2%). In a synthesis
730 of available data from long-term decay studies, Berg et al. (2010) indicated the key role of N and
731 Mn in controlling limit values for plant litter through effects on ligninolytic enzymes; N can
732 suppress late-stage litter decay (Frey et al. 2014), and Mn is required for peroxidase enzymes
733 produced by white-rot fungi. For fine roots dispersed through mineral soil, additional
734 mechanisms involving biophysical inaccessibility are likely.
735 Current evidence indicates that fine roots power forest soil food webs (Pollierer et al.
736 2007, 2012). For example, the most abundant top predator in our north hardwood food web,
737 *Plethodon cinereus* (Burton and Likens 1975), obtained much more ^{13}C through the root channel
738 than the leaf litter channel (Fig. 9). That is, despite the fact that these salamanders do little
739 feeding in soil but confine their feeding activity primarily to litter layers (Fraser 1976), tracer
740 concentrations in salamanders on our litter addition plots was much lower than salamanders from
741 the labeling chambers, suggesting that they derive more carbon through their prey via root
742 sources of carbon compared to leaf sources of carbon. Our tracing of ^{13}C supplied through roots

743 in the labeling chambers also emphasized the importance of herbivory on living roots as an
744 energy source in forest soil food webs: earthworms, root-feeding weevils (*Barypeithes pellucidus*
745 Coyle et al. 2008) and ants that feed on phloem-sucking invertebrates (*Lasius* spp) were the
746 mostly highly enriched taxa collected in litter from the chambers (Fig. 9; Gilbert et al. 2014), all
747 of which are dominant prey in *P. cinereu*s diets (Maerz et al. 2005). Because our sample
748 provided a snapshot in time and only for sapling trees, it is not clear whether the importance of
749 root herbivory for soil food webs is a general feature of these forests. However, we also observed
750 high ^{13}C enrichment in Collembola and Acari (Fig. 9), supporting the argument (Pollierer et al.
751 2012) that basal resources are derived largely from roots (i.e. live roots, root exudates,
752 mycorrhizal fungi, bacteria and saprotrophic fungi).
753 Interest in the role of dead wood in supplying SOM is associated with possible effects of
754 intensive forest harvest for fiber and fuel (e.g., whole-tree harvest) on SOC stocks. Decaying
755 wood can supply SOM via leaching of DOC (Yavitt and Fahey 1985), transport of POC or as
756 residual organic matter on the soil surface. Such incomplete decomposition of branches could be
757 a significant source of SOM in standard, bole-only harvest. However, our observations for small
758 sugar maple wood (2-4 cm) suggests that surprisingly small amounts are stabilized as SOM. In
759 particular, only 0.33% of the ^{13}C in decaying stems was recovered in SOM after six years, when
760 96.2$\pm$0.7% of the wood dry mass had been lost from the decay bags at the THP site (i.e. nearly
761 all the C was mineralized to CO_2). Similarly, at the Arnot site where decay was significantly
762 slower (18.6 % remaining after six years) only 0.14 % was recovered in soil. The difference in
763 decay rate was likely caused by saproxylic invertebrates (*Scarabaeidae*) that were common at
764 the THP site but absent at the Arnot site. Notably, fine POM (partly from invertebrate activity)
765 was five times more abundant in surface soils at the THP than the Arnot site (0.075 % recovery
766 vs 0.015%). These invertebrates also may have affected the wood-rot fungi, a possibility that is
767 currently being evaluated using genomic analysis.
768 In conclusion, research on the transformation and fate of fixed carbon in forest
769 ecosystems has been stimulated by the key role played by forests in the global carbon cycle. Our
770 field studies focused on the effects of invasive earthworms on transport and transformation of
771 leaf litter C in temperate deciduous forest soils. We used ancillary sources of funding to support
772 a suite of complementary studies to take advantage of the expensive isotopic labeling procedure.
773 In particular, we simultaneously measured C flow through the root channel and the leaf litter

774 channel into soils and the soil food web. Because of limited funding for the ancillary studies, the
775 research was somewhat limited in scope and depth; in particular, quantitative budgets could not
776 be constructed. Future work could be improved if equal attention and funding were applied to all
777 the research avenues provided by isotopic labeling. Most important, quantification of net
778 assimilation of the label by the saplings in the chambers would allow calculation of C budgets
779 that would improve comparisons between litter and roots as sources of C for the soil food web
780 and SOM.
781
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787
788 **References**
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Table 1. Earthworm density (A) and ash-free dry biomass (B) in plots with different dominant earthworm assemblages ("Rubellus" dominated by *Lumbricus rubellus*, "Terrestris" dominated by *Lumbricus terrestris*) at Arnot Forest. Values are means across three sampling dates (May 2008, October 2008, October 2009).

A. Density (# per m^2)

PLOT	L. terrestris	L. rubellus	L. immature	Other1)	Total
Rubellus	0	16.3	60.5	29.4	106.2
Terrestris	8.2	7.5	41.3	24.7	81.7

B. Ash-free dry biomass (grams per m^2)

Rubellus	0	1.86	1.58	0.68	4.12

Terrestris	2.91	0.86	2.56	1.39	7.72

1) mostly Octolasion tyrtaeum and Aporrectodea spp.

Figure Captions

Figure 1. Chambers used to label sugar maple saplings growing in the understory of a thinned maple forest with ^{13}C. Note the ports on chamber wall used to scrub CO_2 prior to addition of $^{13}CO2$ label. The plastic has been rolled back from the top and upper half of the chambers and enclosed in shade cloth.

Figure 2. Diagram summarizing the suite of approaches using carbon isotope tracing in sugar maple forest and soil. Five studies that are illustrated refer to the text description in Methods

Figure 3. A. Quadrats for labeled litter additions with garden edging to enclose plots and cover board for accessing salamanders; B. Assembled quadrats viewed from above after one and two years; C. Collection of labeled leaf litter, peeling back litter layer between two screens; D. Sampling underlying soil after removal of forest floor horizons (no earthworm plots)

Figure 4. Mean peak enrichment ^{13}C values of focal biota in soil communities arranged vertically by presumed trophic positions. Width of bars represents enrichment in proportion to basal resources (**bottom panel**: fine roots in field chambers ($124^{0}/_{00}$) ; **top panel**: leaves on forest plots ($323^{0}/_{00}$)). Natural abundance values of ^{13}C are generally $-23^{0}/_{00}$. Only values for microbial community, earthworms, and salamanders are available for forest plots

Figure 5. Recovery of ^{13}C label from leaf litter across three years. Top panel is no earthworm plots, and bottom is earthworm invaded plots. Units are % recovery of added excess

^{13}C in three soil layers (O_i litter, O_{ea} forest floor and mineral soil). Inset boxes are microbial biomass. Note that pathways of loss of ^{13}C from the soil were not directly measured but rather calculated by difference in the mass balance; for example, some of the label loss from surface soils could be associated with particle eluviation rather than gaseous emissions

Figure 6. Soil ^{13}C enrichment (del ^{13}C) across three years and five soil depth increments following addition of ^{13}C labeled leaf litter. Error bars indicate two standard errors (pre-treatment variation too small to appear)

Figure 7. Soil ^{13}C enrichment (del ^{13}C) across three years and five soil depth increments following addition of ^{13}C labeled leaf litter. a) plots dominated by *Lumbricus terrestris* and b) plots dominated by *L. rubellus.* Error bars indicate two standard errors of the mean (pre-treatment variation too small to show up).

Figure 8. Distribution of recovery of added excess ^{13}C (% of total recovery) across five soil aggregate fractions in earthworm invaded and no earthworm plots at Arnot Forest two years after label additions. (a) leaf litter ^{13}C additions and (b) root ^{13}C additions in labeling chambers. Light POM: low-density particulate organic matter; macro: macroaggregates; free micro: microaggregates; occluded micro: microaggregates within macroaggregates. Error bars indicate standard errors

Figure 9. The mean (+/- 1 SE) enrichment of Red-backed salamanders (*Plethodon cinereus*) on forest plots following the application of ^{13}C labeled leaf litter in October of 2007 ("structural label" - see text). Values are relative to salamanders collected outside plots, which were stable over the study duration at $\delta\,^{13}C$ of -22.8 $^{0}/_{00}$. The solid line represents salamanders from forest plots without nonnative earthworms, the fine dashed line and light gray shaded curve represents salamanders from "Rubellus" plots with

1053 invasive earthworm communities that did not include *Lumbricus terrestris,* and the coarse
1054 dashed line and dark gray shaded curve represents salamanders from "Terrestris" forest
1055 plots with invasive earthworm communities dominated by *Lumbricus terrestris.* (see Table
1056 1).
1057
1058 Figure 10. Recovery of ^{13}C label from decaying (a) fine roots and (b) small stem wood, in
1059 five soil aggregate fractions (see Figure 4) in a sugar maple plantation six years after addition to
1060 soils. Error bars indicate standard errors
1061

1062
1063 Fig. 1
1064

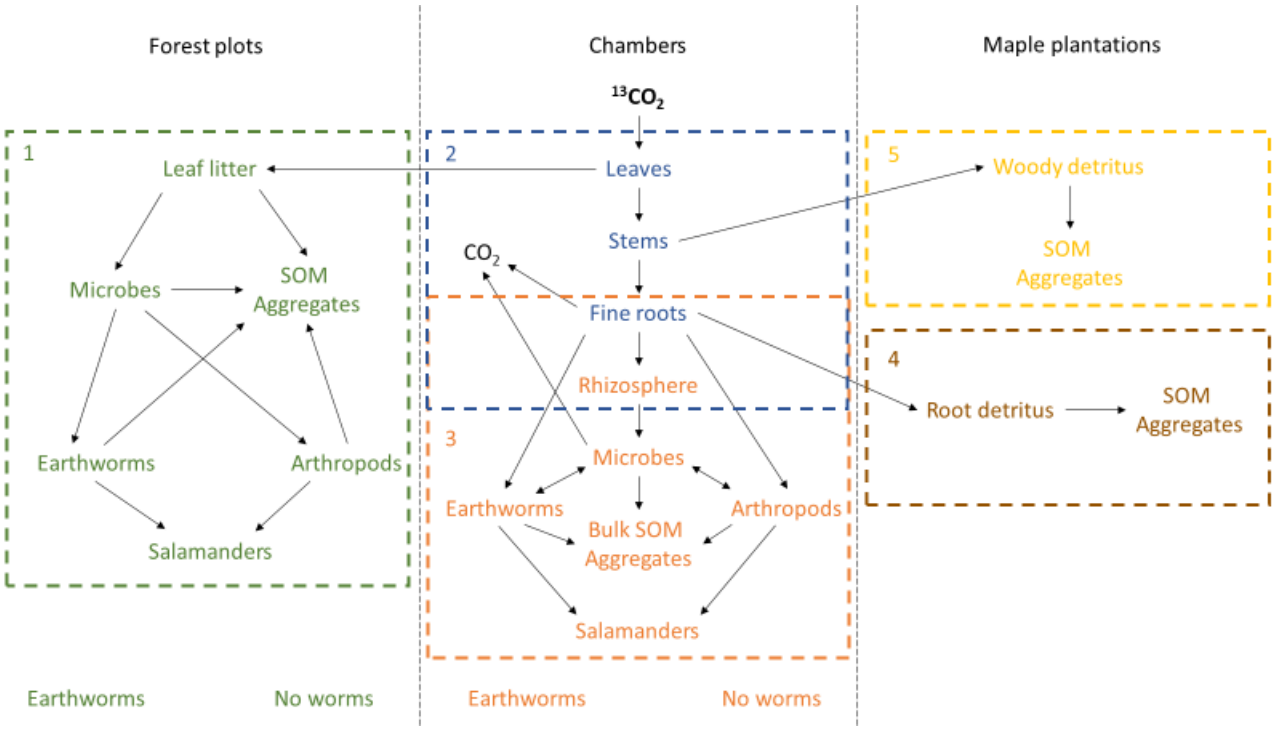

Forest plots
Chambers
Maple plantations
$^{13}CO_2$
1
Leaf litter
Microbes
SOM
Aggregates
Earthworms
Arthropods
Salamanders
2
Leaves
Stems
CO_2
Fine roots
Rhizosphere
3
Microbes
Earthworms
Arthropods
Bulk SOM
Aggregates
Salamanders
5
Woody detritus
SOM
Aggregates
4
Root detritus
SOM
Aggregates
Earthworms
No worms
Earthworms
No worms

Fig. 2

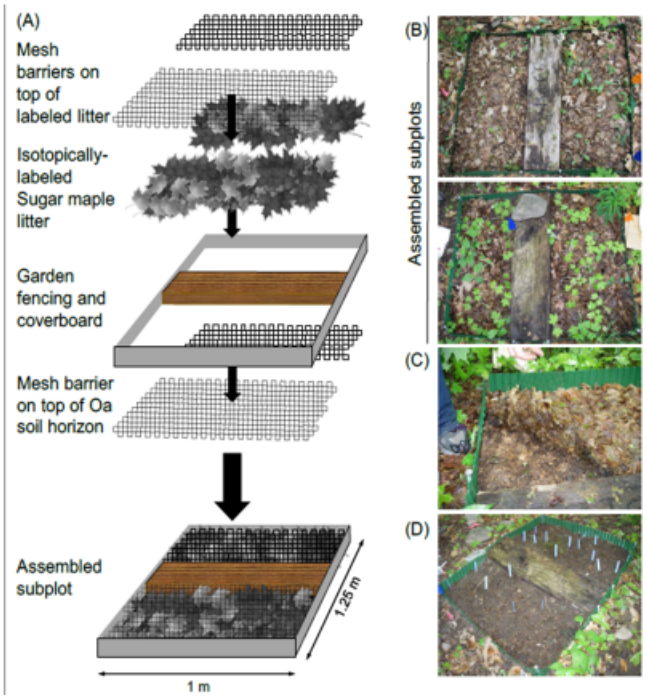

(A)
Mesh barriers on top of labeled litter
Isotopically-labeled Sugar maple litter
Garden fencing and coverboard
Mesh barrier on top of Oa soil horizon
Assembled subplot
1.25 m
1 m
(B)
Assembled subplots
(C)
(D)

Fig. 3

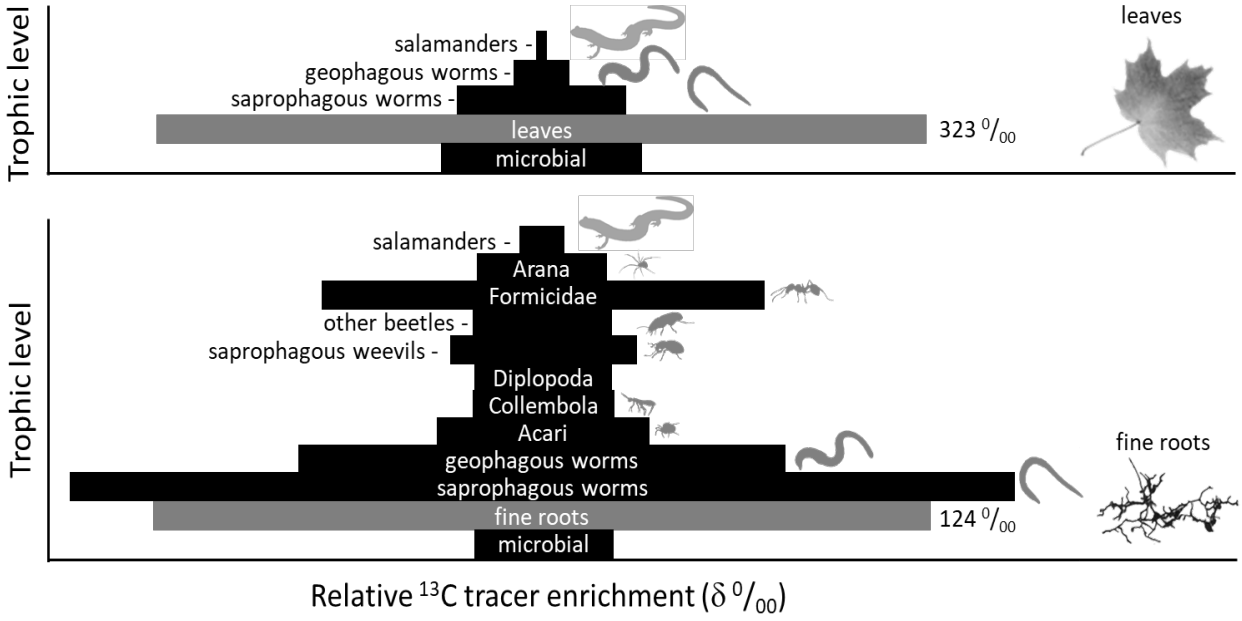

Trophic level
salamanders -
geophagous worms -
saprophagous worms -
leaves
microbial
323 ‰
leaves
Trophic level
salamanders -
Arana
Formicidae
other beetles -
saprophagous weevils -
Diplopoda
Collembola
Acari
geophagous worms
saprophagous worms
fine roots
microbial
124 ‰
fine roots
Relative 13C tracer enrichment (δ ‰)

Fig. 4

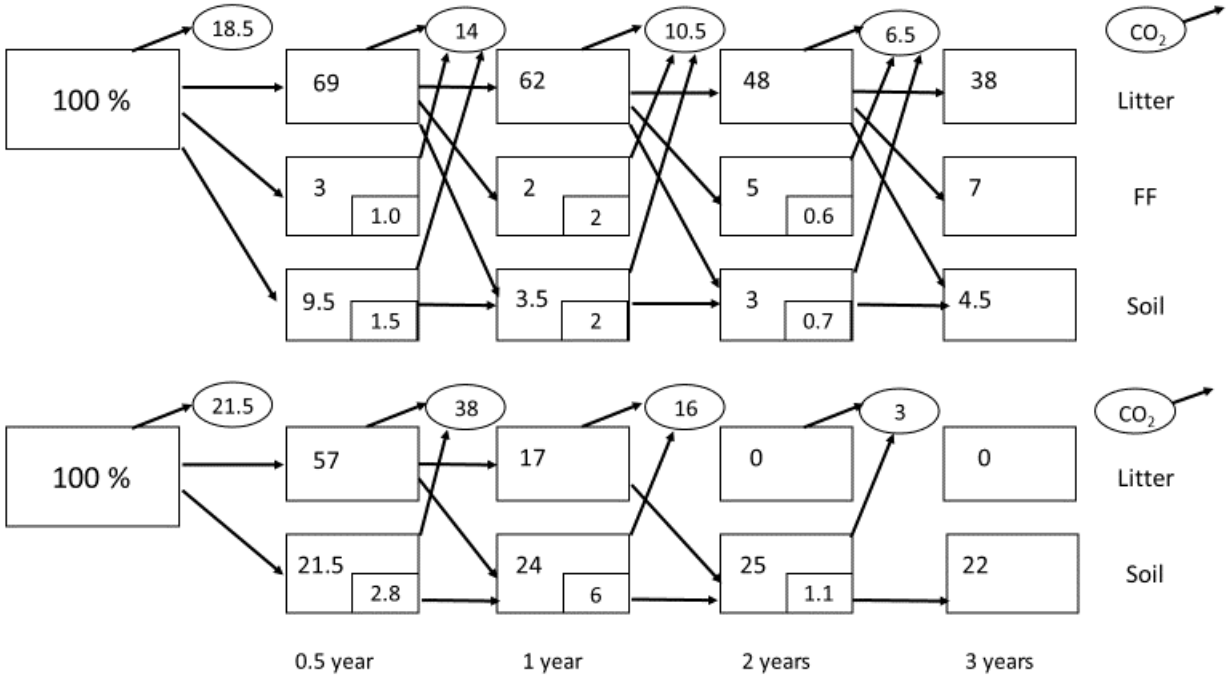
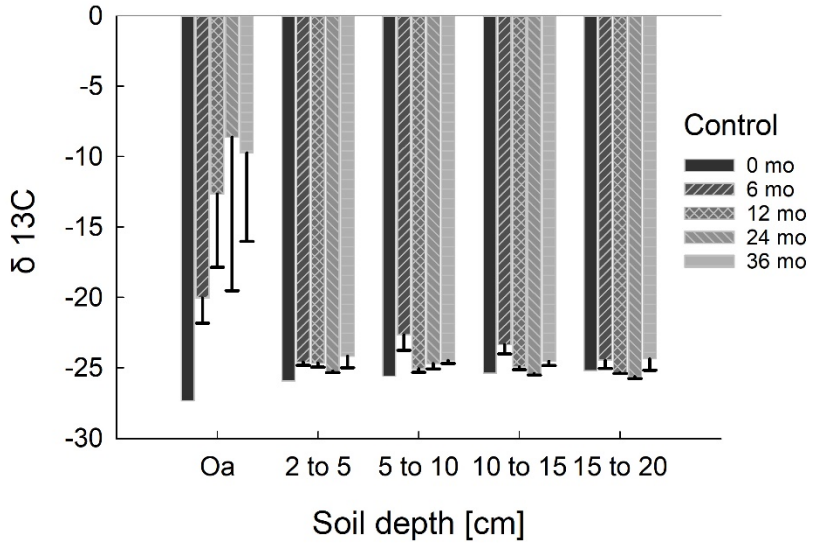
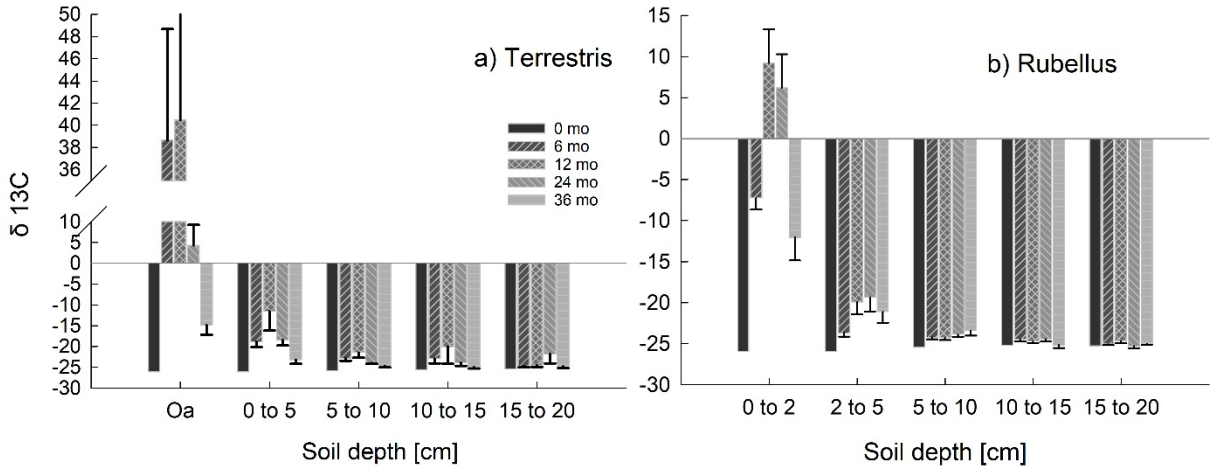

18.5
14
10.5
6.5
CO2
100 %
69
62
48
38
Litter
3
1.0
2
2
5
0.6
7
FF
9.5
1.5
3.5
2
3
0.7
4.5
Soil
21.5
38
16
3
CO2
100 %
57
17
0
0
Litter
21.5
2.8
24
6
25
1.1
22
Soil
0.5 year
1 year
2 years
3 years

Fig. 5


0
-5
-10
-15
-20
-25
-30
δ 13C
Control
0 mo
6 mo
12 mo
24 mo
36 mo
Oa
2 to 5
5 to 10
10 to 15
15 to 20
Soil depth [cm]

1089 Fig. 6
1090
1091



1092
1093 Fig. 7
1094
1095
1096

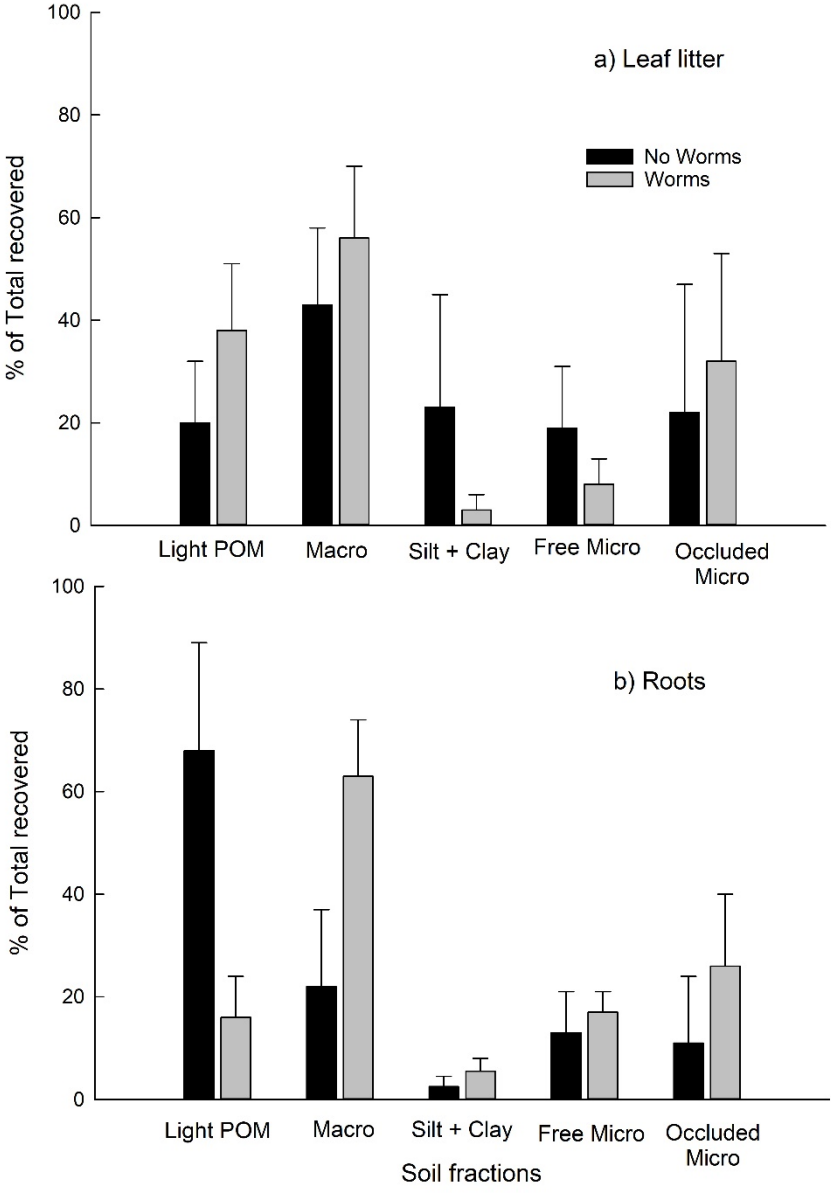

a) Leaf litter
No Worms
Worms
% of Total recovered
100
80
60
40
20
0
Light POM
Macro
Silt + Clay
Free Micro
Occluded Micro
b) Roots
% of Total recovered
100
80
60
40
20
0
Light POM
Macro
Silt + Clay
Free Micro
Occluded Micro
Soil fractions

Fig. 8

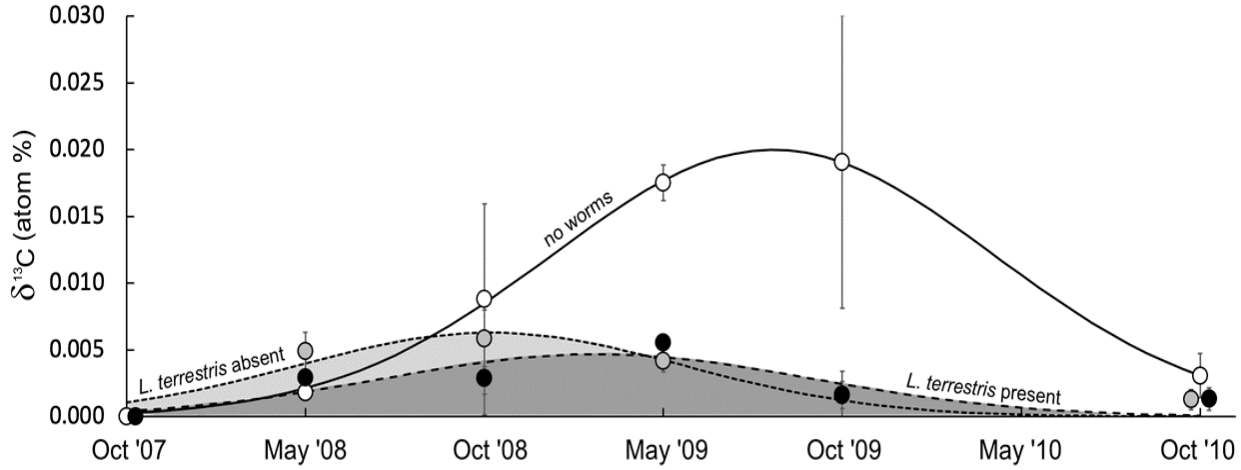

0.030
0.025
0.020
0.015
0.010
0.005
0.000
δ13C (atom %)
no worms
L. terrestris absent
L. terrestris present
Oct '07
May '08
Oct '08
May '09
Oct '09
May '10
Oct '10

Fig. 9

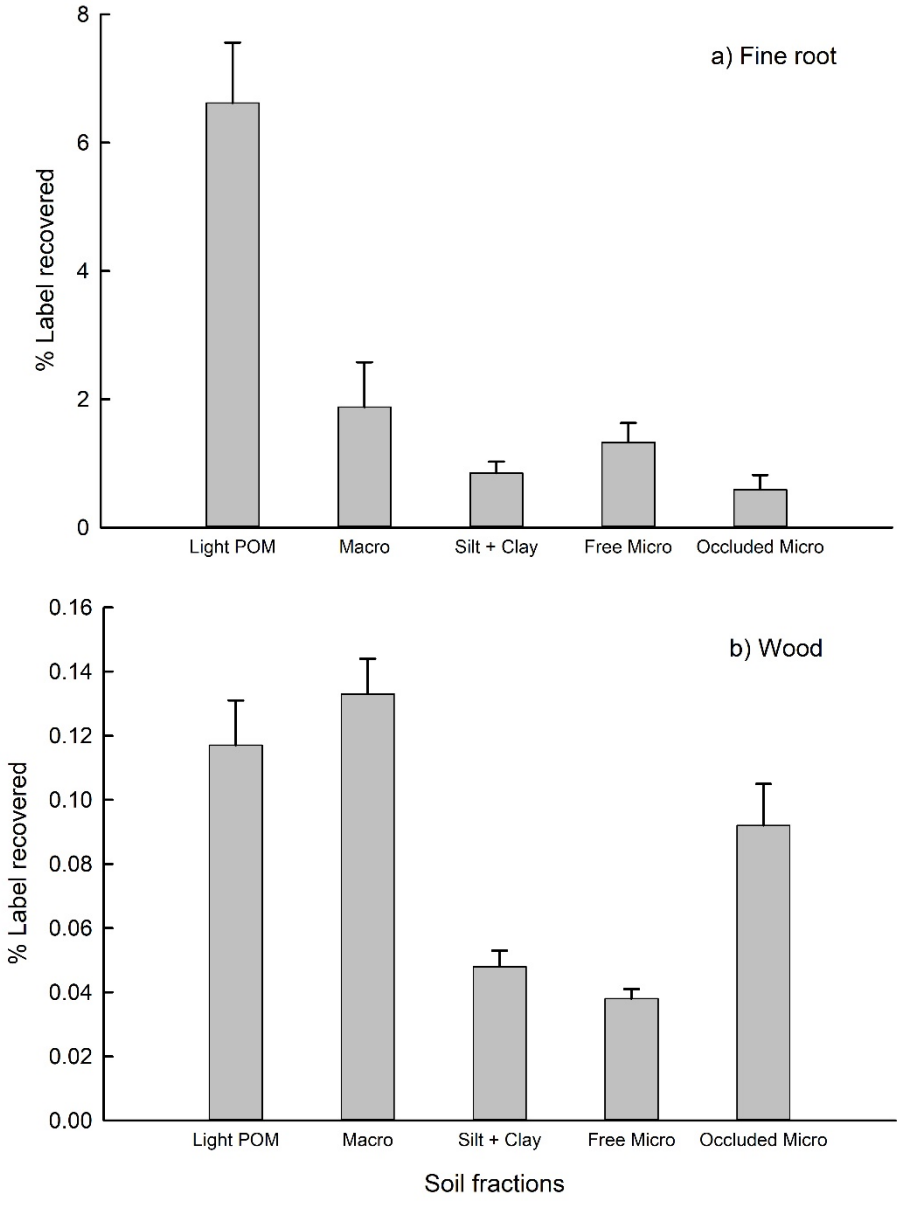

a) Fine root
% Label recovered
8
6
4
2
0
Light POM
Macro
Silt + Clay
Free Micro
Occluded Micro
b) Wood
% Label recovered
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
Light POM
Macro
Silt + Clay
Free Micro
Occluded Micro
Soil fractions

Fig. 10

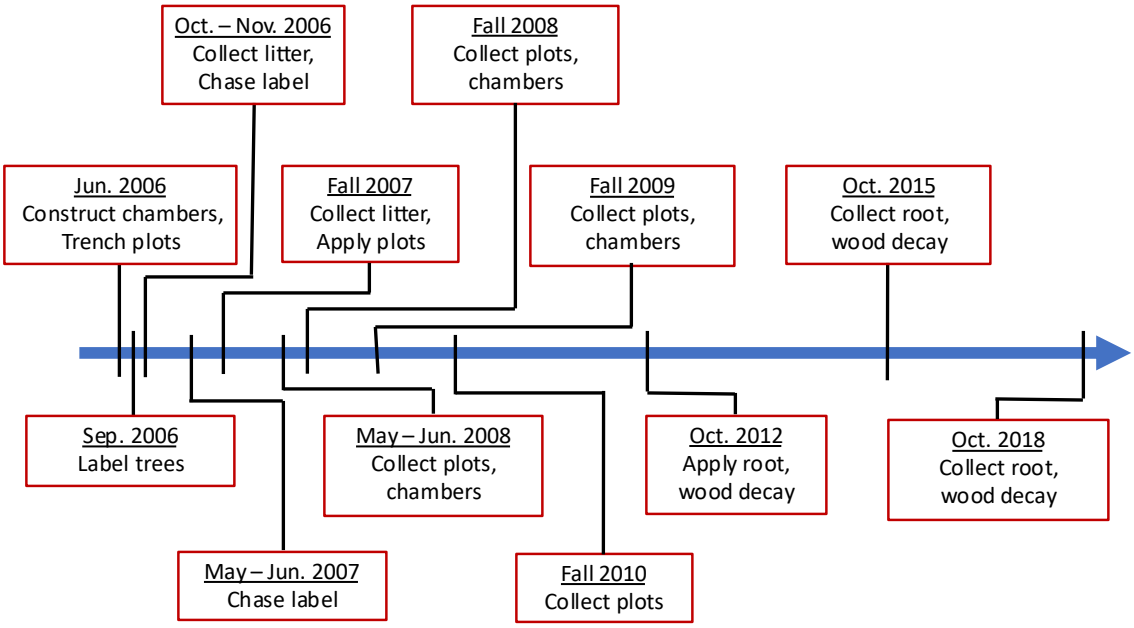


Figure S1. Timeline for the project activities and collections