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Flux of carbon from ^{14}C-enriched leaf litter throughout a forest soil mesocosm

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ABSTRACT

The role of DOC for the build-up of soil organic carbon pools is still not well known, but it is thought to play a role in the transport of carbon to a greater depth where it becomes more stable. The aim of this study was to elucidate within-year dynamics of carbon transport from litter to the O (Oe and Oa) and A horizons. Mesocosms with constructed soil profiles were used to study dynamics of C transport from ^{14}C-enriched (about 1000‰) leaf litter to the Oe/Oa and A horizons as well as the mineralization of leaf litter. The mesocosms were placed in the field for 17 months during which time fluxes and ^{14}C content of DOC and CO_2 were measured. Changes in ^{14}C in leaf litter and bulk soil C pools were also recorded. Significant simultaneous release and immobilization of DOC occurring in both the O and A horizons was hypothesized. Contrary to our hypothesis, DOC released from the labeled Oi horizon was not retained within the Oe/Oa layer. DOC originating in the unlabeled Oe/Oa layer was also released for transport. Extensive retention of DOC occurred in the A horizon. DOC leaching from A horizon consisted of a mix of DOC from different sources, with a main fraction originating in the A horizon and a smaller fraction leached from the overlaying horizons. The C and ^{14}C budget for the litter layer also indicated a surprisingly large amount of carbon with ambient $\Delta^{14}C$-signature to be respired from this layer. Data for this site also suggested significant contributions from throughfall to dissolved organic carbon (DOC) transport into and respiration from the litter layer. The results from this study showed that DOC retention was low in the O horizon and therefore not important for the O horizon carbon budget. In the A horizon DOC retention was extensive, but annual DOC input was small compared to C stocks and therefore not important for changes in soil C on an annual timescale.

1. Introduction

Transport and retention of DOC has been suggested to be an important mechanism for build-up of soil organic C pools in forest soils (e.g. Kalbitz et al., 2005). Due to abiotic retention, e.g. adsorption, there is typically a reduction in DOC flux from approximately 10–40 g m^{-2} under the O horizon to about 1–20 g m^{-2} in the B horizon (Kalbitz et al., 2000, Michalzik et al., 2001). DOC thus retained in the mineral soil is thought to a large extent to be stabilized in slow turnover carbon pools (Kalbitz et al., 2005). Dissolved organic matter has, however, also been suggested to be a significant substrate for soil microorganisms (Marschner and Noble, 2000) and DOC leached from undecomposed substrates has been shown to be especially labile (Kalbitz et al., 2005). In addition, an increasing number of studies suggest that fresh litter does not immediately contribute substantially to stable soil organic matter pools (e.g. Trumbore, 2000, Hagedorn et al., 2003, Swanston et al., 2005). The role of DOC in the long-term accumulation of soil organic matter is thus still widely debated. The aim of this study was to understand the role of DOC in short-term formation and stabilization of soil organic carbon by tracing the movement of enriched radiocarbon from fresh litter through the soil profile.

The Enriched Background Isotope Study (EBIS) took advantage of an unplanned local atmospheric release of ^{14}C from an incinerator near Oak Ridge, Tennessee, USA (Trumbore et al., 2002). That release resulted in a whole-ecosystem isotopic labeling event, which has been used to study the local terrestrial carbon cycle, emphasizing below-ground processes. Results from previous EBIS work showed that movement of carbon from the enriched litter to the mineral soil was limited (Cisneros-Dozal et al., 2007, Fröberg et al., 2007), but did indicate that the ^{14}C was gradually accumulating in the O horizons. After 3 years of ^{14}C-enriched ($\Delta^{14}C \sim 1000‰$) litterfall additions, $\Delta^{14}C$ in the Oe/Oa horizon increased from about 200 to over 300‰. Additionally, ^{14}C-signatures in the enriched litter dropped from approximately 1000‰ to about 700‰ in the year following application of the litter to the field plots. Site-specific modeling of the carbon cycle

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and transport of the ^{14}C-enriched carbon to Oa and A horizons using a litterfall cohort model (P.J. Hanson, unpublished), suggested that retention of DOC leached from the ^{14}C-enriched litter was responsible for much of the ^{14}C accumulation in the Oe/Oa horizon (perhaps 70% over 10 years). From EBIS observations the depletion of ^{14}C in the Oi was hypothesized to result from the disproportionate loss of ^{14}C-enriched compounds through leaching and mineralization of the Oi layer. The field observations for EBIS were limited to an annual time step, which was insufficient to resolve intra-annual dynamics of ^{14}C loss from the Oi horizon and immobilization in the Oe/Oa and mineral soil. To provide higher-resolution data on the dynamics of ^{14}C and mass loss from fresh litter a mesocosm study was executed to quantify within-year dynamics of carbon transport from the Oi litter to the Oe/Oa and A horizons. The following hypotheses were tested:

- Litter-derived CO_2 and DOC during the first months following litter additions would yield ^{14}C-signatures higher than that in the bulk litter due to differences in ^{14}C content in different chemical fractions of the litter (based on observations from the main EBIS study).
- C would be transferred from litter to the O horizon in sufficient quantity to enable detection of changes in ^{14}C-signature in the O horizon at the end of the experiment.
- DOC would be retained within the A horizon, but the amount of DOC-derived ^{14}C from one litter cohort addition would be insufficient to cause a measurable significant change in bulk soil ^{14}C.

2. Methods

2.1. Design of the field experiment

This experiment was conducted on the Oak Ridge Reservation (ORR; 35°58′N; 84°16′E) beneath the canopy of a mature deciduous forest. The area is dominated by an upland oak forest type (*Quercus* spp.; *Acer* spp.) with scattered pine (*Pinus echinata* Mill. and *P. virginiana* Mill.), mesophytic hardwoods (*Liriodendron tulipifera L., Fagus grandifolia* J.F. Ehrh.), and some hickory (*Carya* spp.). Mean annual temperature is 14 °C and mean annual precipitation is 1358 mm.

The experiment was performed using mesocosms with combinations of homogeneous soil and humus material with the addition of fresh *Quercus prinus* L. litter. All mineral soils, humus materials and ^{14}C-enriched fresh litter materials used to construct the mesocosms were collected and manipulated within the ORR. The constructed soil profiles in the different mesocosm treatments were designed to approximate different depths in typical soil profiles found at the Oak Ridge reservation (Fig. 1). The three types of mesocosms are abbreviated as L, LO and LOA, respectively, throughout the rest of the paper.

- The L treatment contained 15.7 g dry matter (DM) of air-dried ^{14}C-enriched chestnut oak (*Q. prinus* L.) litter (Oi horizon) collected at Pine Ridge in autumn 2000, equal to an annual input rate of 500 g dry matter m^{-2}. Initial mean ^{14}C-signature of the added litter was 953 ± 22‰.
- In addition to the fresh litter, the LO treatment mesocosms contained 31.4 g (DM) of field moist Oe/Oa horizon, corresponding to 1000 g DM m^{-2}. This O horizon was collected at Walker Branch on the Oak Ridge Reservation.
- Finally, the LOA mesocosms contained the same amount of ^{14}C-enriched litter and O horizon soil, plus 2.74 kg (DM) of surface mineral soils (characterized as the A horizon) corresponding to 86 kg m^{-2}. The A horizon soil was collected at Haw Ridge on the Oak Ridge Reservation from a Typic Paleudult, Fullerton series, with a kaolinitic mineralogy. More details about the soil may be found in Johnson et al. (2007, 2008). The mineral soil was gently compacted to occupy a 10 cm depth to approximate the mean A-horizon bulk density of the field soils of the Oak Ridge Reservation.

Eight sets of mesocosms were constructed for harvesting through time. Each set of consisted of 5 replicates of each mesocosm type (L, LO, LOA) for a total of 120 individual mesocosms.

2.2. Mesocosm construction

Mesocosms were constructed using 20 cm diameter PVC pipe. At the bottom of all mesocosms 700 g of glass beads (Potter Industries A170 glass spheres, US sieve no. 10–14, diameter 1.4–2.0 mm) were added in order to get a flat surface with good drainage. Bead size was chosen to provide continuous solid-to-solid contact avoiding soil–air interface to minimize perching of water in the A horizon soil. To facilitate subsequent sampling of distinct layers, polyethene screen (1.5 mm square mesh) was added above the glass beads, between all layers of the mesocosms and above the ^{14}C-enriched litter added to the top of each mesocosm. Natural litterfall was regularly removed from the surface of the mesh screen throughout the experiment.

At the start of the experiment, the defined C stocks in the three layers were 235 g C m^{-2} in ^{14}C-enriched litter, 390 g C m^{-2} in the Oe/Oa

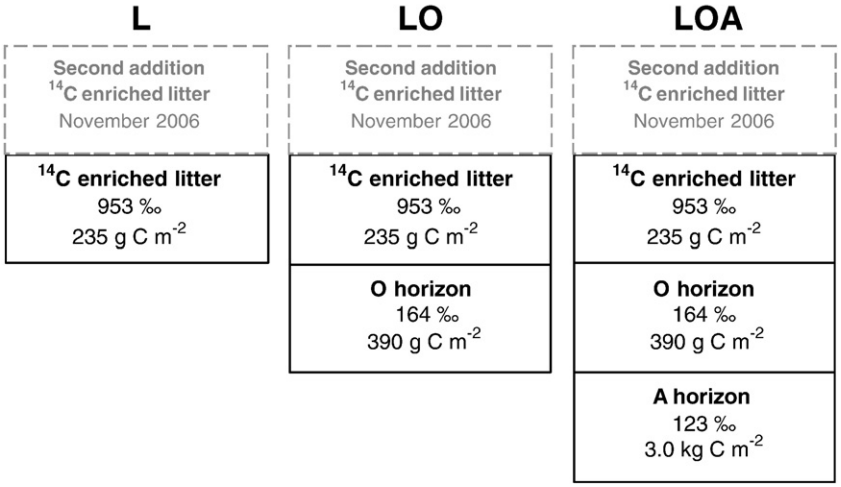

Fig. 1. Schematic representation of the three different treatments with initial conditions for $\Delta^{14}C$ (‰) and C stocks (g C or kg C m^{-2}). The ^{14}C-enrichment level was the same for both litter additions. Mesocosm treatments are L = litter only; LO = litter plus organic humus and LOA = litter plus organic humus plus A-horizon mineral soils.

horizon, and 3.0×10^3 g C m^{-2} in the A horizon (Fig. 1). Mesocosms without the litter additions were placed in the field in February 2006 to saturate and infiltrate the O and A material with natural rainfall. The ^{14}C-enriched litter was added to all mesocosms 6 weeks later in March of 2006, and again in November 2006 to the unharvested mesocosms. The second litter addition at the same level as the first litter addition provided another pulse of ^{14}C to each mesocosm to achieve a stronger ^{14}C pulse and provided a second opportunity to study the dynamics of C release from fresh leaves. To track changes in C, and ^{14}C over time, five replicates from each of the mesocosm treatments were destructively sampled and all horizons separated and sampled. This was done in April, June, August, November of 2006 and February, May and August of 2007.

2.3. Soil solution sampling

Soil solutions passing through all mesocosm layers were sampled by event over the whole experiment. In total, 51 events were sampled. Precipitation per event ranged from 7 to 70 mm precipitation (with a mean of 24 mm) per event. Precipitation at the experimental site (especially summer events) occurs as convectional rainfall in distinct events. Soil solution collections were typically done the day after a major rainfall. Soil solution was allowed to drain freely at the base of the mesocosms and collection was made in glass bottles. Soil solution was sampled from 5 replicates of each mesocosm type for each sampled event. DOC concentrations were converted to flux values by multiplying DOC concentration data by the associated measured water fluxes. Reported variations in DOC fluxes were based on the variances of measured DOC concentrations assuming identical water inputs into all mesocosms.

The DOC draining from the L, LO and LOA mesocosms was analyzed for all defined precipitation events. The ^{14}C analysis of collected soil solutions, however, was based on pooled event sampling between defined harvest dates. Solutions from individual mesocosms of the same type were subsampled on a volume-weighted basis and pooled to represent the DOC flux for the defined sampling period. The ^{14}C in throughfall was only evaluated during three periods of measurements distributed throughout the experiment.

2.4. CO_2 and $^{14}CO_2$ efflux

Respiration measurements from duplicate L, LO and LOA mesocosms were evaluated using autochambers following the methods of Czimczik et al. (2006). Autochambers were running during the first week of manipulations in March 2006 to capture the initial CO_2 loss from the additions of ^{14}C-enriched litter, but they were removed and used for another study from April to August 2006. In August of 2006 they were returned to service in the EBIS project for 12 months from August 2006 to August 2007. Although not continuous, the available CO_2 efflux measurements for the mesocosms provided a full annual cycle which included observations through the second addition of ^{14}C-enriched litter. CO_2 respired from the mesocosms was well correlated with air temperature (all treatments) and litter water content (L and LO treatments). These relationships were used in period-specific linear regression models for gap filling the CO_2 data, when measured data was not available. Models were optimized by minimizing the residuals between measured and modeled data for the specific time period.

CO_2 evolved from mesocosms for ^{14}C analysis was collected in molecular sieve traps from the L and LOA mesocosms near the midpoint between harvests using methods described by Cisneros-Dozal et al. (2007). LO mesocosms were excluded for budget reasons. Lids were placed over the top of the mesocosms and initial CO_2 removed from headspace air by circulating it through a soda lime trap. CO_2 concentrations were then allowed to build up to levels of several hundred parts per million, after which air was dried (using calcium sulfate) and CO_2 trapped at ambient temperature on an activated 13X molecular sieve. Molecular sieve traps were sent to the University of California, Irvine (UCI) where CO_2 was released and the traps reactivated for further use by baking at 610 °C. The released CO_2 was purified cryogenically and converted to graphite using the Zn reduction method (Xu et al., 2007). An aliquot of each ^{14}C sample was analyzed for ^{13}C using continuous flow isotope ratio mass spectrometry at UCI. Approximately 0.1 µl of purified CO_2 was removed from the vacuum line with a syringe and injected into a He-flushed septum-capped vial. The isotopic signature of the CO_2 was measured using a Gas bench II inlet to a Delta-plus stable isotope mass spectrometer.

Low rates of CO_2 evolution and the high porosity of the glass beads supporting the mesocosm layers suggest that the CO_2 trapped from mesocosm headspace was a likely combination of both CO_2 evolved from decomposing organic matter in the mesocosms and ambient air CO_2. The difference in the ^{13}C between air samples (~−8‰) and CO_2 derived from decomposition (assumed to be equal to ^{13}C measured in bulk litter (~−28‰) were used to estimate the fraction of CO_2 from background air in the sample (F) using the following equation:

$$F=\left(\delta^{13}C_{sample}-\delta^{13}C_{decomp}\right)/\left(\delta^{13}C_{air}-\delta^{13}C_{decomp}\right),$$

where $\delta^{13}C_{sample}$ is the $\delta^{13}C$ in the CO_2 sample and $\delta^{13}C_{decomp}$ and $\delta^{13}C_{air}$ are $\delta^{13}C$ in samples with 100% sample and ambient air respectively.

The radiocarbon signature of the heterotrophically respired CO_2 was then calculated from mass balance and measured $\Delta^{14}C$ of CO_2 in background air:

$$\Delta^{14}C_{decomp}=\left(\Delta^{14}C_{sample}-F*\Delta^{14}C_{air}\right)/(1-F),$$

where $\Delta^{14}C_{sample}$ is the $\Delta^{14}C$ in the in the CO_2 sample and $\Delta^{14}C_{air}$ is the $\Delta^{14}C$ in ambient air, measured at the site.

To avoid arbitrary corrections, data for $^{14}CO_2$ efflux were not estimated when the calculations suggested that F was greater than 0.5 (i.e. the sample CO_2 was more than 50% derived from background air). In practice, F values lower than 0.5 were achieved for 90% of data.

2.5. Laboratory assessment of litter-derived $^{14}CO_2$

Laboratory incubations of the *Quercus* litter used in the mesocosms were performed to collect litter-derived ^{14}C for comparison to the $\Delta^{14}C$-signature of CO_2 respired in the L only mesocosms. Roughly 30 g of leaf litter were suspended in aluminum foil in a 1 L glass mason jar with moistened glass beads as described in Cisneros-Dozal et al. (2007). Duplicate samples were incubated at room temperature (24–27 °C), with stopcocks open to air to avoid buildup of CO_2 concentrations beyond 3%. Prior to sampling for isotopes the jars were flushed with CO_2-free air, sealed and CO_2 concentrations allowed to build up to > 1% CO_2. They were then attached to vacuum lines and CO_2 cryogenically purified and analyzed for ^{13}C and ^{14}C content. Jars were sampled ~1 week and 5 months after the incubation started.

2.6. Analytical approaches

Dry weight of remaining litter layer (Oi) was measured for all mesocosms at each harvest. The C and N concentrations and ^{14}C content of the litter layer was determined for each LOA mesocosm at each harvest. To save on experimental costs the ^{14}C-content of the mineral soil was determined at every second harvest because previous studies and modeling work suggested that bulk changes in this horizon would be slow to develop. Samples were analyzed for total C and N on a LECO CN-2000 (LECO Corporation, St. Joseph, Michigan) using secondary standards traceable to NIST reference materials. DOC

concentration was analyzed for each collection using a Shimadzu 5050 TOC analyzer. Prior to all analyses, solutions were filtered (0.45 μm).

Radiocarbon values were measured on the Van de Graaff FN accelerator mass spectrometer (AMS) at the Center for Accelerator Mass Spectrometry, Lawrence Livermore National Laboratory, Livermore California and the W.M Keck Carbon Cycle AMS at UC Irvine (respiration samples). In preparation for AMS analysis, samples were combusted in evacuated, sealed tubes in the presence of CuO and Ag, then reduced to graphite coating on iron powder in the presence of H_2 (Vogel et al., 1984). Splits of combusted sample were taken for ^{13}C analysis from each organic and mineral horizon for correction of mass-dependent fractionation in the reported radiocarbon values, and all radiocarbon values are presented as $\Delta^{14}C$ (‰) according to Stuiver and Polach (1977).

2.7. Environmental data

Air temperature, soil temperature, litter water content (methods according to Hanson et al., 2003) and soil water content (ECH_2O, Decagon Devices, Inc.) were recorded for randomly assigned mesocosms. Those data were used to develop models of CO_2 fluxes during periods when CO_2 measurements were not made. These data are not shown, but are available from the authors.

2.8. C mass balance calculations

Calculations of C budgets are based on soil C stocks, DOC fluxes and CO_2 fluxes, in combination with ^{14}C data. The ^{14}C data is used to estimate the fraction of a carbon flux or pool derived from different substrates and this information is used in simple mass balance calculations. For all calculations we assume that DOC and CO_2 have the same ^{14}C concentrations as the substrate from which they were derived.

For soil solutions the fraction of DOC that originated from the above lying horizons was calculated as:

$$F_{above} = \left(DO^{14}C_{horizon} - SO^{14}C_{horizon}\right) / \left(DO^{14}C_{above} - SO^{14}C_{horizon}\right),$$

where $DO^{14}C_{horizon}$ is the ^{14}C in DOC leached from the horizon of interest (i.e. DOC from the LO treatment if we are interested in the O horizon), $SO^{14}C$ is the ^{14}C of soil organic carbon in the same horizon. $DO^{14}C_{above}$ is the ^{14}C in DOC leached from the horizon above (i.e. from the L treatment, if we are studying the O horizon).

Similarly, the fraction of CO_2 from litter in the L treatment was calculated as

$$F_{litter} = \left({}^{14}CO2_L - {}^{14}C_{Throughfall}\right) / \left({}^{14}C_{litter} - {}^{14}C_{Throughfall}\right),$$

where $^{14}CO2_L$ is ^{14}C in CO_2 from the L treatment, $^{14}C_{Throughfall}$ is annual average ^{14}C in throughfall and $^{14}C_{litter}$ is ^{14}C in litter in the mesocosm.

2.9. Statistical analysis

Tests for statistical significance (α=0.05) of changes over time were done using *t*-tests, comparing conditions at the start of the experiments with conditions at the end of the experiments. Standard error of the mean is used to indicate variability (±) throughout the manuscript.

3. Results

3.1. C stocks

Mass loss from the ^{14}C-enriched litter differed between mesocosm treatments (Fig. 2). Total C loss from both ^{14}C-enriched litter cohorts at the end of the experiment was highest for the LO treatment with a total loss of 191±22 g C m^{-2} followed by the LOA treatment with a loss of 154±32 g C m^{-2} (Table 1). C loss from ^{14}C-enriched litter in the L treatment was lower at only 118±19 g m^{-2}. Nitrogen stock in ^{14}C-enriched litter did not change and consequently the C/N ratio in the ^{14}C-enriched litter added to LOA treatment in March 2006 dropped from 99 in March 2006 to 43 in August 2007.

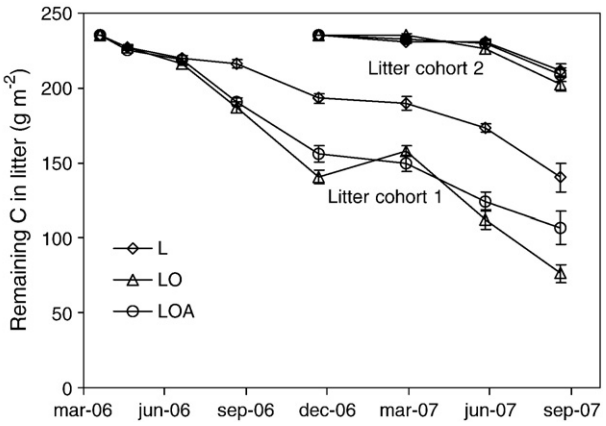

Fig. 2. Changes in C stocks (g C m^{-2}) in the annual Oi cohorts in the different treatments. Litter was added in March (litter cohort 1) and November (litter cohort 2) 2006. Error bars represent standard error. Mesocosm treatments are as defined for Fig. 1.

Even though screens were present to allow uniform subsampling of all horizons (L, O, and A), the mobility of the humus layer (in the absence of anchoring root systems) made it unreliable to sample the mass of O horizon in a consistent way at each harvest. Therefore, C mass balance of this horizon was not obtained.

In the A horizon there was a tendency (p=0.08) for decreasing C concentration (Fig. 3). Initial C concentration at the start of the experiment was 3.5±0.1% and C concentration at the end of the experiment 3.3±0.1%. The corresponding C stocks were 3.0±0.1×10^3 g C m^{-2} and 2.8±0.1×10^3 g C m^{-2}, respectively.

3.2. DOC

Highest DOC concentrations were measured in the LO treatment, with on average 63 mg DOC L^{-1}, followed by the LOA treatment with average DOC concentration 39 mg L^{-1} (averaged over time of the whole experiment). The solution leached from the L treatment had a concentration of 32 mg DOC L^{-1} averaged over the whole experiment. Renewed addition of ^{14}C-enriched litter in November 2006 led to a significant increase in mean DOC concentrations for the L treatment from 22 mg L^{-1} before to 47 mg L^{-1}after the second litter addition. In the other two treatments changes in concentrations after renewed litter addition were more moderate, 57 and 67 mg L^{-1} for LO and 36 and 42 mg L^{-1} for LOA before and after litter additions, respectively. Average DOC concentration in throughfall was 12 mg L^{-1}. DOC concentrations in all treatments followed the seasonal pattern observed in many other studies (Kalbitz et al., 2000) with high concentrations during summer and lower concentrations during winter. Total fluxes of DOC throughout the experimental period were 12, 36, 61 and 45 g m^{-2} for throughfall, L , LO and LOA, respectively (Fig. 4).

3.3. CO_2

Total fluxes based on the summation of measured and model-interpolated data (Fig. 5) were 207, 338 and 498 g C for the L, LO and LOA treatments, respectively, implying that CO_2 fluxes were approximately 5–10 times higher than the corresponding DOC fluxes from the same treatments. Of these totals, 27, 39 and 40% of the combined flux estimate were based on model interpolated data for the L, LO and LOA treatments respectively.

Table 1
Total carbon fluxes±SE in g C m^{-2} over the duration of the study (17 months) for each form of the mesocosm

Type of mesocosm C flux	Mesocosm treatment		
	L	LO	LOA
Litter mass loss	118±19	191±22	154±32
DOC flux	36±7	61±12	45±7
CO_2 flux	207	338	498

L=litter only; LO=litter plus organic humus; LOA=litter plus organic humus plus A-horizon mineral soils.

3.4. ^{14}C

For the fresh litter cohorts, the $\Delta^{14}C$ decreased, from 953±22 to 890±10‰ over 17 months for the first litter cohort (p=0.02) and from 975±12 to 926±11‰ (p=0.01) over 6 months for the second litter cohort (Fig. 6). The $\Delta^{14}C$-signature in the O horizon did not change significantly (p=0.33) during the course of the experiment and was 164±3‰ and 167±6‰ at the start and at the end of the experiment, respectively (Fig. 6). The A horizon also showed no significant change with values of 123±5‰ and 129±7‰, respectively (p=0.21) (Fig. 6). The pattern of increasing ^{14}C levels for both the O and A horizons over time is consistent with some net litter-to-soil DOC transport.

3.5. $DO^{14}C$

The $\Delta^{14}C$ in DOC leached from the L treatment was for most periods significantly lower than the $\Delta^{14}C$ in the ^{14}C-enriched leaf material. $DO^{14}C$ from the L treatment varied between approximately 500 and 900‰ (Fig. 7). In the LO treatment changes in $DO^{14}C$ approximately paralleled the $DO^{14}C$ in the L treatment for most observations, but with lower ^{14}C, ranging from about 300 to 700‰ (Fig. 7). ^{14}C in DOC from the LOA treatment had a different temporal pattern than the L and LO treatments. Initially the $\Delta^{14}C$ in DOC was close to $\Delta^{14}C$ in the soil, but gradually increased to approximately 200–400‰ during the last 12 months of the experiment (Fig. 7). Average throughfall $\Delta^{14}C$ measured in 2006 was 315±3‰ (based on two periods of sampling), higher than what should be expected from present levels of ^{14}C in the atmosphere. A large release of ^{14}C was recorded on the Oak Ridge Reservation in 2006 similarly to the original 1999 release as demonstrated by ^{14}C sampling of the local atmospheric CO_2 on Walker Branch (Hanson and Trumbore, unpublished data) that could have contributed to these high and possibly temporally variable ^{14}C inputs. For 2007 (based on one period of sampling) $\Delta^{14}C$ in throughfall was 174±18‰.

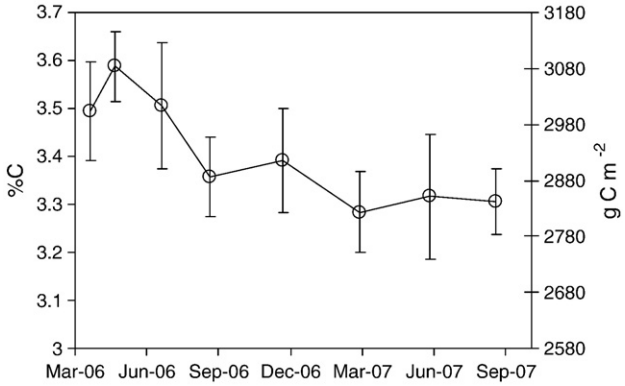

Fig. 3. Changes in C concentration (%) and C stock (g C m^{-2}) in the A horizon of the LOA treatment. Error bars represent standard error.

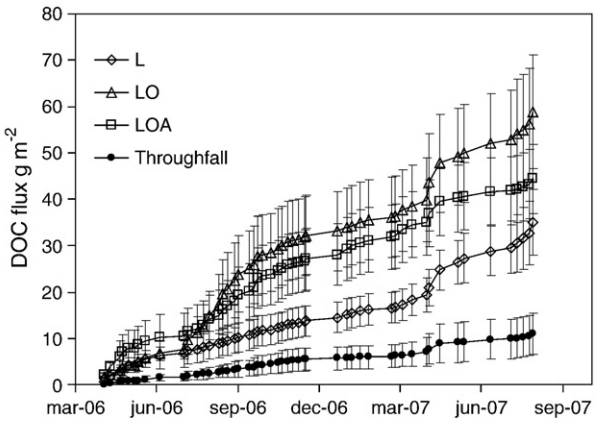

Fig. 4. Cumulative DOC fluxes (g C m^{-2}) in the three different treatments and throughfall. Errors bars represent standard error. Mesocosm treatments are as defined for Fig. 1.

3.6. $^{14}CO_2$

$\Delta^{14}C$ in respired CO_2 from the L treatment varied with time, ranging from about 300 to 1100‰ (Fig. 8) and was typically lower than the $\Delta^{14}C$-signature of the enriched litter but close to $\Delta^{14}C$ in DOC. In February of 2007, the average ^{14}C in CO_2 from the L treatment was slightly higher than in bulk ^{14}C-enriched litter. The $^{14}CO_2$ in the LOA treatment was also similar to $DO^{14}C$ from the same treatment with $\Delta^{14}C$ of about 200 to 400‰ (Fig. 8). LO treatment $^{14}CO_2$ data were not obtained (see Methods).

3.7. Litter lab incubations

The CO_2 respired from litter incubated in the laboratory was initially enriched in ^{14}C. During the first days of incubations average $\Delta^{14}C$ in CO_2 was 1095‰ (two replicates: 1082 and 1108‰), which was higher than in solid litter (just under 1000‰). After nearly 5 months of incubation, however, the $\Delta^{14}C$ in CO_2 had decreased to 903‰ (two replicates: 871 and 935‰) (p=0.02).

4. Discussion

Fluxes of carbon in each of the three mesocosm treatments are summarized in Fig. 9. The data presented in this paper suggested that retention of DOC in the O horizon, contrary to our hypothesis, was not

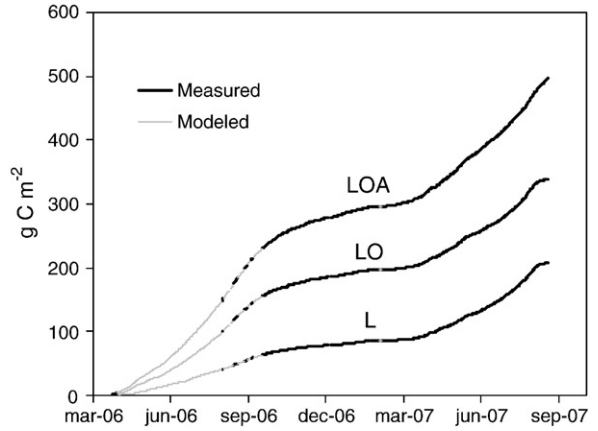

Fig. 5. Cumulative efflux of CO_2 (g C m^{-2}) from the three mesocosms. Measured data are dark points and model interpolations for the 2006 season are added based on measured litter and soil temperatures and moisture. Mesocosm treatments are as defined for Fig. 1.

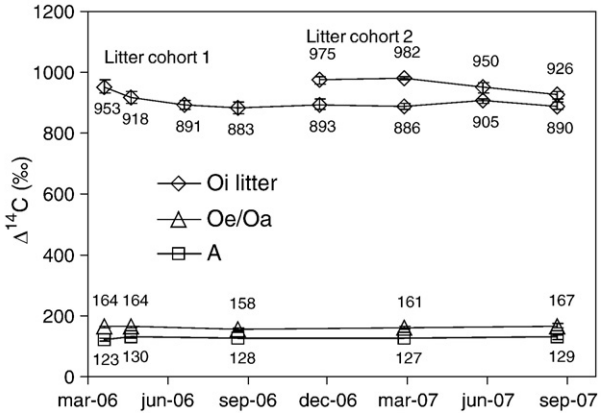

Fig. 6. $\Delta^{14}C$-signature of Oi, Oe/Oa and A horizons from the LOA mesocoms. Error bars (typically within symbol sizes) represent standard error.

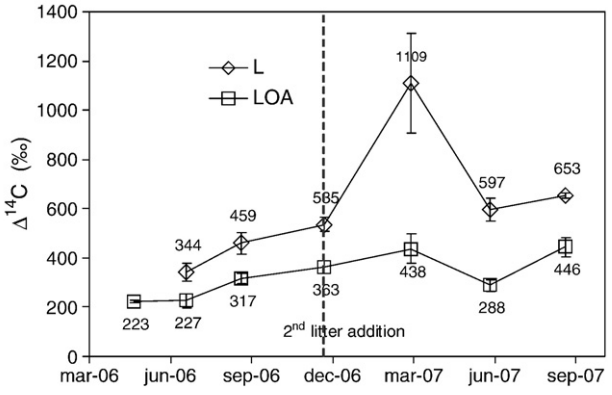

Fig. 8. $\Delta^{14}C$ in CO_2 from the L and LOA treatments. Error bars represent standard error. No measurements were made for the LO treatment. Mesocosm treatments are as defined for Fig. 1.

significant and that extensive retention and release of DOC occurred in the A horizon. The C and ^{14}C budget for the litter layer also indicated that a surprisingly large amount of carbon with ambient $\Delta^{14}C$ signature was respired from this layer. These observations will be discussed below.

4.1. Low DOC retention in the O horizon

We originally hypothesized that there would be a significant retention of DOC in the O horizon. The extent of DOC retention in organic horizons in general is currently poorly known. Qualls (2000) reported that adsorption dominated over desorption in a forest floor, i.e. there was decrease in DOC concentration, but only at DOC concentrations of at least 700 mg L^{-1}. It has also been suggested (Guggenberger and Kaiser, 2003; Kleber et al., 2007) that much of the retention of DOC in mineral soils occurs to organic matter and not to mineral surfaces. However, as will be discussed below, there were no indications of extensive sorption of DOC in the O horizon in our study. ^{14}C measurements of DOC and ^{14}C measurements of soil both suggested small retention of litter-derived DOC in the O horizon.

Higher litter mass loss from the LO and LOA treatments compared to the L treatment made budget calculations in the LO treatment and the O layer difficult. Nevertheless, $\Delta^{14}C$ in leachate from the LO treatment showed that a significant fraction of the DOC released from the litter was transported through the O horizon and down to the A horizon. The $\Delta^{14}C$ in DOC leaving the LO treatment gradually increased from approximately 300–400‰ during the first months of the experiment to relatively stable values of about 500–600‰ during the second half of the experiment (Fig. 7). A simple mass balance, based on ^{14}C data, indicated that about 50% of DOC leached from the LO treatment originated in ^{14}C-enriched litter and that the remainder of the DOC was derived from throughfall or from the O horizon, which both had similar ^{14}C-signatures (Fig. 7). Total flux of DOC from the LO treatment was 61 g m^{-2} (Table 1) and consequently about 30 g of DOC that originated from ^{14}C-enriched Oi passed through the Oe/Oa horizon. Total flux of DOC from the L treatment was 36 g m^{-2} (Table 1), of which about 15 g, according to ^{14}C measurements, was derived from sources other than the ^{14}C-enriched litter. Paradoxically, a budget calculation suggests that more DOC with origin in ^{14}C-enriched litter was leached from the LO treatment than from the L treatment. However, DOC leaching from ^{14}C-enriched litter in the LO treatment was likely greater than in the L treatment, as reflected by the higher mass loss from litter in the LO treatment. The budget of DOC is therefore not possible to constrain. Nonetheless, the $\Delta^{14}C$ of the Oe/Oa horizon did not change significantly, implying that DOC retention from ^{14}C-elevated Oi inputs was small.

The ^{14}C measurements of soil in the O horizon also suggest low retention of litter-derived DOC in the O horizon. A change in O horizon soil $\Delta^{14}C$ of approximately 10‰ at the end of the experiment would be statistically significant ($p<0.05$), which with an initial C stock of about 400 g and $\Delta^{14}C$-signature of about 164‰, would require retention of only approximately 5 g of DOC with ^{14}C-signature equal to that in the litter. The change in ^{14}C in the O horizon was however non-significant, increasing only from 164‰ to 167‰.

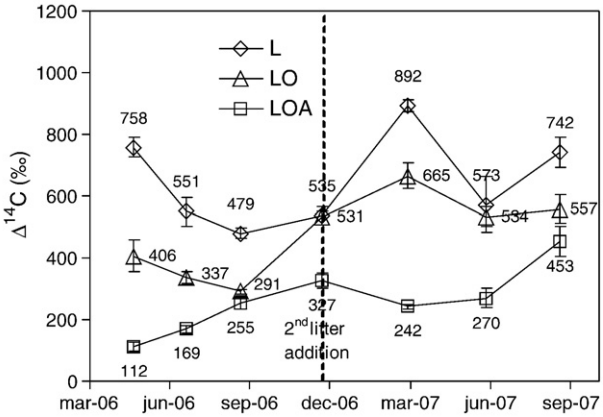

Fig. 7. $\Delta^{14}C$ in DOC from the three treatments. Error bars represent standard error. Mesocosm treatments are as defined for Fig. 1.

4.2. Extensive exchange of DOC in the A horizon

There was a trend of decreasing C concentration in the A horizon over the course of the experiment. This was expected, because normal inputs of root litter to the soil were precluded in the mesocosm work. From fluxes of CO_2 and DOC data, it was estimated that net loss in the A horizon with initial C stock of 3.0 kg was about 0.14 kg C m^{-2}; based on soil C concentration data the estimated decrease was 0.16 kg C m^{-2}. Although the A horizon lost carbon through mineralization, it was still a net sink of DOC. The data from the LO treatment showed that a total of 61 g DOC entered the A horizon from the O horizon and a total of 45 g DOC was leached from the LOA treatment during the experiment, resulting in a net retention of 16 g DOC in the A horizon. Assuming that $\Delta^{14}C$ in DOC from the A horizon was equal to $\Delta^{14}C$ in bulk soil, it can be estimated by mass balance calculations that 27 of 45 g DOC in leachates from the LOA treatment had its origin in the A horizon, and 18 g from the overlying horizons (Oi and Oe/Oa). About 44 g of the 62 g of DOC leached from the O horizon was thus retained in the A horizon. The different temporal patterns in DO^{14}C compared to the L and LO

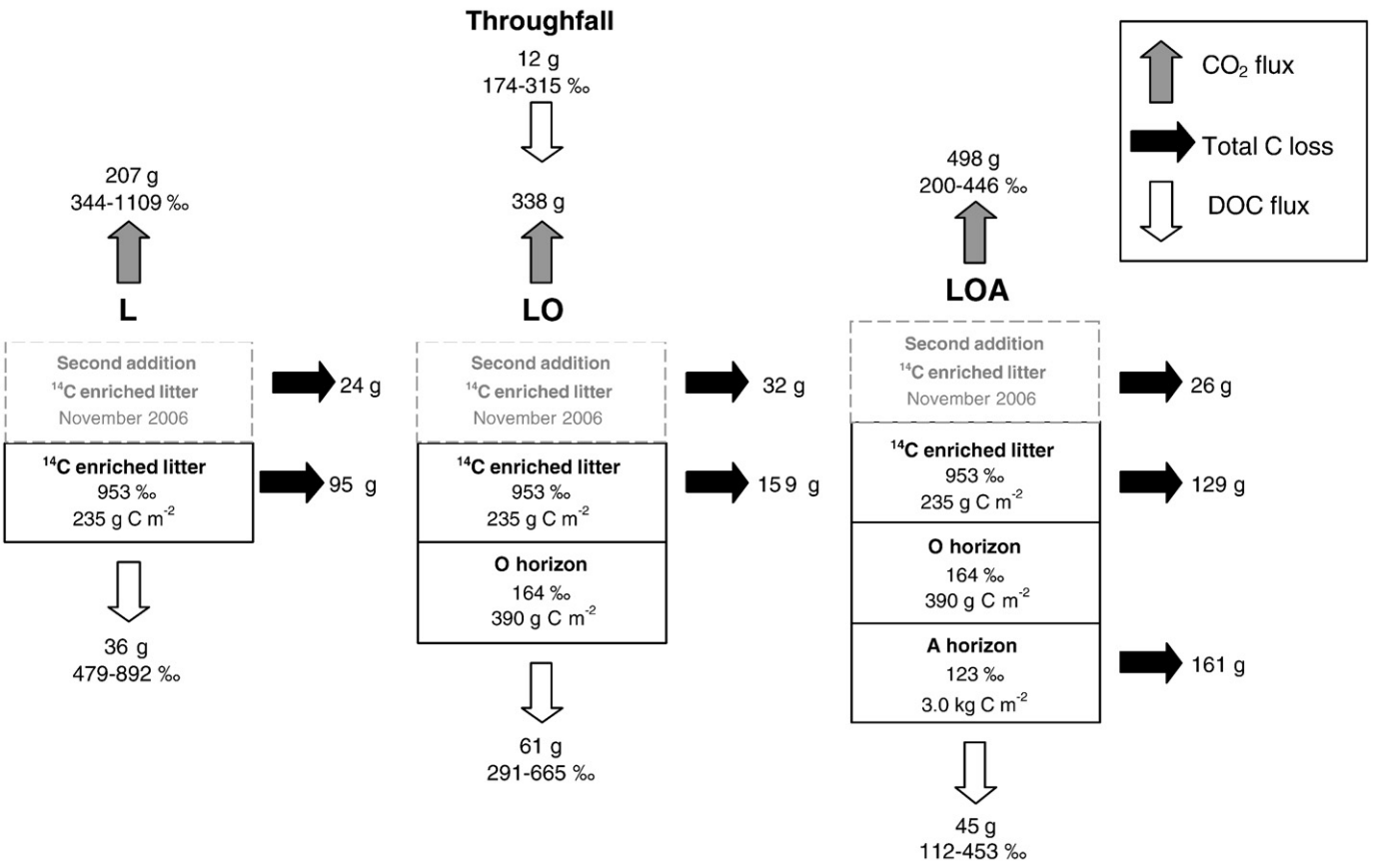

Fig. 9. Summary of $\Delta^{14}C$ and C fluxes from the different treatments (L, LO, LOA as defined in Fig. 1) designated by the arrow styles shown in the legend in including total CO_2 efflux from the mesocosm surface, DOC flux from the mesocosm, and total C loss by treatment and horizon or litter cohort combination. Data are for the entire 17-month experimental period. The range of throughfall $\Delta^{14}C$-signatures represents a limited number of sampling events and the mean or median values are not well known.

treatment also indicate that exchange (i.e. both retention and release of DOC) was extensive in the A horizon. This seems to be a general phenomenon, which occurs in many different soil types with different mineralogy. Hagedorn et al. (2002) and Fröberg et al. (2007) showed that DOC captured in mineral soil is derived only to a minor extent from fresh surface litter.

The gradual increase in $\Delta^{14}C$ signal in DOC from the LOA treatment suggests that there is simply a time lag of DOC accumulation in mineral soils, with the pool of carbon with origin in fresh litter equilibrating with carbon from the A horizon over longer time periods. ^{14}C data of PLFAs in the A horizon of the LOA treatment sampled in August 2006 (Trumbore, unpublished data) indicated that DOC from ^{14}C-enriched litter was not a significant substrate for microorganisms in the mineral soil, suggesting that litter-derived DOC delivered to and retained in the mineral soil was not rapidly mineralized by established soil microorganisms.

Mass balance calculations suggest that the higher ^{14}C in incoming DOC from the O horizon compared to DOC leached from the A horizon would have increased the $\Delta^{14}C$ in soil with about 5‰, which was not large enough for a statistically significant change. This is in agreement with observations that showed no significant change in A horizon $\Delta^{14}C$ (Fig. 6).

4.3. Significant contributions from non-litter sources to DOC and CO_2 from the L treatment

Surprisingly, both DOC and CO_2 in the L treatment had significantly lower $\Delta^{14}C$ than bulk ^{14}C-enriched litter, suggesting that there were major contributions (approximately 50%) from non-litter sources to both DOC and respiration from this horizon.

The low $\Delta^{14}C$-signature of DOC leached from the L treatment (Fig. 7) could be explained by dilution from DOC with lower ^{14}C-signature in throughfall, whereas this seemed like a less likely explanation for the low $\Delta^{14}C$ in CO_2 (Fig. 8). A mass balance of sources of DOC was calculated, assuming that $\Delta^{14}C$ in DOC from the ^{14}C-enriched litter had the same ^{14}C-signature as bulk litter and that the remainder of the DOC in the L treatment had a ^{14}C-signature equal to the $\Delta^{14}C$ in throughfall. Using these assumptions, about 60% or 21 g m^{-2} of the 36 g DOC m^{-2} from the L treatment originated in the ^{14}C-enriched litter and the remaining 15 g m^{-2} from throughfall, which is in reasonable agreement with the measured DOC flux of 12 g m^{-2} in throughfall.

The $\Delta^{14}C$ in DOC and respiration from both the L and LOA treatments largely followed the same temporal pattern during the experiment (Figs. 7 and 8). However, whereas contribution from throughfall was a reasonable explanation for low $\Delta^{14}C$ in the L treatment, C from throughfall was not a likely explanation for the low $\Delta^{14}C$ in respired CO_2 from the L treatment. The flux of DOC in throughfall was too small to sustain a dilution of ^{14}C in the respiration from the mesocosms. The $\Delta^{14}C$ data suggested that close to 50% or 98 g m^{-2} of the 207 g of respired CO_2 from the L treatment was derived from carbon with ambient or close to ambient $\Delta^{14}C$, assuming that CO_2 had $\Delta^{14}C$-signature similar to that in bulk ^{14}C-enriched litter. This is more than 8 times the amount of C measured in throughfall.

Although throughfall is known to have high concentrations (in some cases >50%) of labile fractions of DOC (e.g. Qualls and Haines, 1992; Yano et al., 2000), indicating that it may partly be mineralized before DOC analysis and thus never recorded as a DOC flux, the amount of labile C in throughfall needed to explain the low $\Delta^{14}C$-signature in DOC and respired CO_2 has not been reported in the literature. The ^{14}C data are also in agreement with data of CO_2 fluxes and mass loss from

litter, showing small C loss in litter compared to total CO_2 and DOC efflux from the litter (Fig. 9). Mass loss data showed that C loss from ^{14}C-enriched litter in the L treatment was only 118 g C m^{-2} (Table 1), which can be compared to the total CO_2 flux of 207 g m^{-2} and the total net DOC leaching of 24 g m^{-2}, implying that total DOC and CO_2 losses were twice as high as litter mass loss, which match the estimates of an approximate 50% contribution from non-litter sources to CO_2 from the L treatment. Furthermore both DO^{14}C and $^{14}CO_2$ peaked, with $\Delta^{14}C$ equal or higher than bulk ^{14}C-enriched litter, during winter when the canopies were all bare. It seems difficult to account for this large contribution from non-litter sources to DOC leaching and respiration, be it DOC in throughfall or small particulate organic matter from the canopy or something else. We have not been able to find a satisfactory explanation for our observations, but overall the data in this paper are internally consistent and both signatures of ^{14}C in DOC and CO_2 and their temporal variations and litter mass loss data tell the same story.

Higher than expected $\Delta^{14}C$-signature in throughfall during 2006 (315‰), may be explained by local releases of ^{14}C, similar to those in 1999–2000. Such releases were detected through air-monitoring stations (Trumbore and Hanson, unpublished data), but were not strongly reflected in litterfall collected in the fall of 2006, which had an average $\Delta^{14}C$-signature of 176‰, which is also higher than the ambient atmospheric $\Delta^{14}C$.

The calculations here rely on the assumption that $\Delta^{14}C$ in CO_2 and DOC were similar to that in bulk ^{14}C-enriched litter or bulk soil. The laboratory studies, however, showed that CO_2 has, at least during the early stages of decomposition, a higher $\Delta^{14}C$ than the residue fraction, which may be explained by different ^{14}C-concentrations in different leaf chemical constituents. Because the differences are fairly small compared to the difference between the ^{14}C-enriched litter and the other sources of C such a bias does not change the general picture that emerged from the field experiment. Low mass loss from ^{14}C-enriched litter in the L treatment compared to the other two treatments is another factor that makes it necessary to interpret the data carefully. This was likely caused by drier conditions and lower resulting microbial activity due to absence of moist soil or humus coming in contact with the L material. Mass loss rates in LO and LOA were approximately equal to litter mass loss rates measured at Walker Branch watershed by Hanson et al. (2003, 2005).

5. Conclusions

1. DOC derived from the litter layer was not to a significant degree retained in the O horizon.
2. Extensive retention of DOC occurred in the A horizon. DOC leaching from the A horizon consisted of a mix of DOC from different sources, with a main fraction originating in the A horizon and a smaller fraction leached from the overlaying horizons.
3. A surprising amount of C respired from litter and leached as DOC was derived from a small size fraction litter inputs (DOC in throughfall etc.).

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