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Recent (<4 year old) leaf litter is not a major source of microbial carbon in a temperate forest mineral soil

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ABSTRACT

Microbial communities in soil A horizons derive their carbon from several potential sources: organic carbon (C) transported down from overlying litter and organic horizons, root-derived C, or soil organic matter. We took advantage of a multi-year experiment that manipulated the ^{14}C isotope signature of surface leaf litter inputs in a temperate forest at the Oak Ridge Reservation, Tennessee, USA, to quantify the contribution of recent leaf litter C to microbial respiration and biomarkers in the underlying mineral soil. We observed no measurable difference ($<\sim$40‰ given our current analytical methods) in the radiocarbon signatures of microbial phospholipid fatty acids (PLFA) isolated from the top 10 cm of mineral soil in plots that experienced 3 years of litterfall that differed in each year by $\sim$750‰ between high-^{14}C and low-^{14}C treatments. Assuming any difference in ^{14}C between the high- and low-^{14}C plots would reflect C derived from these manipulated litter additions, we estimate that $<\sim$6% of the microbial C after 4 years was derived from the added 1–4-year-old surface litter. Large contributions of C from litter < 1 year (or >4 years) old (which fell after (or prior to) the manipulation and therefore did not differ between plots) are not supported because the ^{14}C signatures of the PLFA compounds (averaging 200–220‰) is much higher that of the 2004–5 leaf litter (115‰) or pre-2000 litter. A mesocosm experiment further demonstrated that C leached from ^{14}C-enriched surface litter or the O horizon was not a detectable C source in underlying mineral soil microbes during the first eight months after litter addition. Instead a decline in the ^{14}C of PLFA over the mesocosm experiment likely reflected the loss of a pre-existing substrate not associated with added leaf litter. Measured PLFA $\Delta^{14}C$ signatures were higher than those measured in bulk mineral soil organic matter in our experiments, but fell within the range of ^{14}C values measured in mineral soil roots. Together, our experiments suggest that root-derived C is the major (>60%) source of C for microbes in these temperate deciduous forest soils.

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1. Introduction

Microbes in soil A horizons derive their nourishment from several potential carbon (C) sources: downward C transport from overlying litter and O horizons, and locally derived C from roots, rhizosphere fungi, recycled microbial C, or soil organic matter. Recent studies taking advantage of differential isotope labeling of these sources in forest soils suggest that downward transport of litter C is not a major source of C in soil A horizon microbial biomarkers (Bird et al., 2008; Brant et al., 2006a,b), heterotrophically respired CO_2-C (Cisneros Dozal et al., 2006), or soil organic matter C sources (Fröberg et al., 2007; Hanson et al., 2005; Swanston et al., 2005).

Complexities associated with the vertical transfer of dissolved C within soil profiles, especially significant sorption and desorption within the O horizon, alters the nature and age of DOC that enters the mineral soil (Fröberg et al., 2003), and may add a time lag to delay the appearance of litter-derived C in A horizon organic matter for up to several years. In cultivated soils, both plant and soil organic matter derived C have been shown to be important contributors to microbial biomarker compounds, but active mixing of litter forms during cultivation make it impossible to separate above-and below-ground plant contributions (Kramer and Gleixner, 2008). Nonetheless, a picture is emerging in which root-derived C is apparently the predominant source of mineral soil

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organic matter in forest soils (Bird and Torn, 2006; Brant et al., 2006b; Swanston et al., 2005).

The Enriched Background Isotope Study (EBIS) takes advantage of the production of ^{14}C-enriched leaf and root tissues following a large release of radiocarbon by a local hazardous waste incinerator in the summer of 1999 (Trumbore et al. 2002). In 2000, enriched leaf litter was collected and then used to set up manipulation plots that for the next three years received either enriched ('high-^{14}C', or ~980‰) or less enriched ('low-^{14}C', or ~215‰) leaf litter instead of natural litterfall. High-^{14}C or low-^{14}C litter was applied to replicate plots located in the eastern and western sections of the Oak Ridge Reservation (ORR). These sites were in areas either closer to (western) or farther from (eastern) the hazardous incinerator that was the source of the ^{14}C label in 1999. Vegetation at those divergent locations experienced different levels of labeling of root and soil organic matter prior to the controlled EBIS manipulations (Fröberg et al., 2007; Gaudinski et al., 2009; Hanson et al., 2005; Joslin et al., 2006). The EBIS experimental plots were set up in a replicated experimental design for combinations of high-^{14}C and low-^{14}C labeling of below-ground (i.e. east versus west locations) and above-ground (leaf litter manipulation) C inputs. Previous publications resulting from the EBIS experiment have used this experimental design to demonstrate the importance of the leaf litter as a variable source of soil respired CO_2 (Cisneros Dozal et al., 2006); the use of the label to infer root longevity and dynamics (Joslin et al., 2006; Gaudinski et al. 2009; Riley et al., 2009), and the contribution of leaf litter as a source of DOC (Fröberg et al., 2007) and soil organic matter (Swanston et al. 2005).

Using the high-^{14}C leaf litter, Fröberg et al. (2009) used mesocosms with constructed soil profiles to study the shorter-term (<1 year) dynamics of leaf litter mineralization and DOC transport to the Oe/Oa and A horizons. That study showed that DOC was isotopically altered (but not retained) in the O horizon, and that some of the high-^{14}C C derived from surface litter was sequestered in the A horizon. There was no measurable change in the ^{14}C of A horizon soil C, however, as the overall DOC input to the A horizon was small compared to C stocks. Mass balancing of both C and ^{14}C inputs and losses suggested that sequestration of DOC in the A horizon would have increased the $\Delta^{14}C$ in soil organic matter by only about 5‰. Such a small change was not large enough to detect (Fröberg et al., 2009).

In this study we report measures of radiocarbon in microbial respiration and specific biomarker compounds to detect how much leaf litter-derived C was utilized by the microbial community in the uppermost 0–10 cm of mineral soil. Three years of high-^{14}C versus low-^{14}C litter additions at EBIS plots resulted in only small differences between plots in the radiocarbon signature of bulk mineral soils to 15 cm depth (Fröberg et al., 2007; Swanston, et al., 2005; J. Jastrow and C. Swanston, personal communication). However, much of the bulk organic matter is in forms that turn over slowly, and may therefore mask changes in more labile C fractions in the total C pool. By isolating isotopic signatures of C representing only the microbial pool and of the soil's C sources, we expected to limit interferences from slower cycling soil organic matter pools and therefore more easily detect differences in microbial ^{14}C derived from incorporation of high-^{14}C versus low-^{14}C litter C. For this study we use two measures of the isotopic signatures for microbial C sources: the isotopic signature of heterotrophically respired CO_2 as measured in incubations, and the isotopic signature of biomarkers extracted from living microbial cell walls.

Phosphlipid Fatty Acids (PLFA) are compounds found in living microbial cell walls and are frequently used as characteristic biomarkers for microbial community structure (see review by Leckie, 2005). After microbial cell death the phospholipids degrade quickly in soil and thus the PLFAs are assumed to represent active microbial biomass carbon sources. After microbial cell death the PLFAs degrade quickly in soil and thus are assumed to represent active microbial biomass C sources. Carbon isotopic signatures of PLFA have been used to identify microbial C sources in a number of studies (Bird et al., 2008; Brant et al., 2006b; Denef et al., 2009; Kramer and Gleixner, 2006; Moore-Kucera and Dick, 2008; Rethemeyer et al., 2004; Rubino et al., 2009; Waldrop et al., 2000; Waldrop and Firestone, 2006). Our hypothesis was that the PLFAs present in living microbes would show greater influence of leaf litter as a microbial C source than the bulk or low-density organic matter sampled from the same soils. We also hypothesized that individual PLFA compounds (biomarkers specific to cell walls for gram-positive, gram-negative bacteria or fungi) would differ in the incorporation of the labeled leaf litter substrates (Kramer and Gleixner, 2006, 2008). Here we report results of compound specific radiocarbon measurements on PLFA biomarkers collected after 3 years of manipulation of leaf litter ^{14}C values at the EBIS sites. Because the timing of our study took place one year following the period of active litter manipulation, we also took advantage of the mesocosm experiment (Fröberg et al., 2009) that traced sources of ^{14}C-labeled litter over the initial months following enriched litter additions.

2. Methods

2.1. Soil sampling – long-term experimental (EBIS) plots

We sampled mineral soils from manipulation plots maintained by the EBIS experiment on the ORR at the Oak Ridge National Laboratory in Tennessee, USA (361 N, 841 W; Fröberg et al., 2007; Gaudinski et al., 2009; Hanson et al., 2005; Swanston et al., 2005). The four sites differed in their distance from the source of the 1999 ^{14}C release and therefore in the ^{14}C-enrichment in roots and pre-2000 leaf litter. Experimental plots both adjacent and away from the ^{14}C source were established on both Ultisols and Entisol soils. At all four sites, eight replicate 7x7 meter plots were established for leaf litter manipulation experiments. Litterfall was manipulated in the autumn-winter of 2001–2002, 2002–2003, and 2003–2004. In each of these years, natural litterfall was excluded by laying tarps over the sites in October-November. Stored and dried leaf litter collected in 2000 was added in February (2002) or January (2003, 2004). The added leaf litter had ^{14}C contents ranging from +950–990‰ ('high-^{14}C' plots) or +210–220‰ (low-^{14}C litter plots); half of the plots received high-^{14}C and half low-^{14}C litter. For each soil type (Ultisol and Entisol), we therefore had treatments with all combinations of high-^{14}C and low-^{14}C root inputs (eastern versus western reserve sites) and high-^{14}C and low-^{14}C leaf litter inputs (manipulation plots).

In this paper PLFA data from two sites in the eastern reserve are presented. The two sites are referred to as: Walker Branch (WB; Ultisol) and Haw Ridge (HR; Entisol). These sites were chosen because similarities in the ^{14}C signatures of roots and the low-^{14}C label leaf litter in the western reserve sites limited our abilities to distinguish these two as sources of respired CO_2 (Cisneros Dozal et al., 2006). Samples for PLFA analysis were collected from the mineral soil (0–10 cm depth) in 2005, after the termination of litter addition treatments on these plots. In the autumn of 2004, natural leaf litter was allowed to fall into the plots. Litterfall radiocarbon in that year had ^{14}C values of +110–120‰ at Haw Ridge and Walker Branch. These values are higher than the background atmosphere in 2004, reflecting either ^{14}C residual in plant storage pools (Gaudinski et al., 2009), and/or additional ^{14}C releases at the sites. The collected PLFA samples allowed us to contrast the ^{14}C signatures of PLFA from soils having similar pre-manipulation levels of root ^{14}C inputs and background soil organic matter ^{14}C, but which also experienced three years of either high-^{14}C or low-^{14}C leaf litter, plus a final year where litter inputs were not different.

A second measure of microbial C sources was obtained by measuring the ^{14}C signature of CO_2 respired in soil incubations. We sampled the Ultisol plots in 2002, 2003 and 2004 growing seasons at both eastern and western reserve locations (Walker Branch and TVA sites, respectively). Replicate soil cores (0–5 cm depth, 5 cm diameter) were collected 2–3 times during the growing season from the high-^{14}C and low-^{14}C litter treatment plots and incubated intact to determine the ^{14}C signature of heterotrophically respired CO_2 as described in Cisneros Dozal et al. (2006). Data are reported both for the Walker Branch litter treatment plots in the eastern ORR and the Ultisol plots located west of the ORR (i.e., TVA plots; Hanson et al. 2005) but closer to the incinerator source of the ^{14}C.

2.2. Mesocosm experiments

Soils collected in 2005 for PLFA measurements from the long-term field manipulation plots reflect several years of manipulated (high- or low-^{14}C) litter additions followed by a return to litter that had the same ^{14}C signature across all plots. To address the degree to which litter that is <1 year old can influence PLFA radiocarbon, we also measured changes in the ^{14}C of A horizon PLFA in experimental mesocosms set up in 2006 (Fröberg et al., 2009). The mesocosm experiments were designed to study intra-annual transformations of DOC derived from leaf litter in O and A horizons (Fröberg et al., 2009). Soil to construct the mesocosms was collected from the eastern part of the ORR. Mesocosms (20 cm diameter PVC pipe) were constructed to have a leaf litter layer (L; 15.7 g dry weight comprised of oak leaf material collected in 2000 with $\Delta^{14}C$ of $+953 \pm 22‰$). The enriched litter was placed over an Oa horizon (31.4 g dry weight with bulk $\Delta^{14}C$ of $164 \pm 3‰$) and an A horizon (2740 g dry weight with bulk $\Delta^{14}C$ of $123 \pm 5‰$). The L, O, and A horizons were separated by fine mesh screens and stacked over glass beads to allow collection of percolating DOC. Mesocosms were placed out-doors and sampled periodically for fluxes and isotopes of DOC, CO_2, and C in soils. We report analyses of PLFA from combined samples of the A horizons from mesocosms sampled in March and November 2006, just before the addition of litter and 8 months after litter addition respectively; other data from the mesocosm experiments are reported in Fröberg et al. (2009).

2.3. Laboratory analyses

2.3.1. Incubations

Incubation procedures are described in Cisneros Dozal et al. (2006). Briefly, one core (5 cm diameter, integrating 0–5 cm depth) from each of three replicate plots per treatment (low-^{14}C litter addition and elevated-^{14}C litter addition) was collected 2–3 times per year from the Ultisol plots. Cores, with intact roots, were allowed to sit for up to one month in a refrigerator (to allow roots to die and therefore minimize root respiration), then were placed in a 0.5 l glass jar with an air-tight lid fitted with two stopcocks to allow for air purging and sampling. Incubations were performed at field moisture and water was added to the jar to ensure 100% relative humidity. The jar headspace was purged with CO_2-free air, and then sealed. CO_2 was allowed to accumulate until sufficient concentrations (up to 1–3% CO_2) were present for measurement of radiocarbon. An aliquot of the jar headspace was removed using a pre-evacuated container; jar headspace was replaced with CO_2-free air to maintain atmospheric pressure in the jar. The CO_2 in the sample was then purified cryogenically and reduced to graphite targets for AMS radiocarbon analysis as described in (Xu et al., 2007).

2.3.2. PLFA analysis

Roughly 1 kilogram of soil was sampled for PLFA extraction, purification and analysis. For the long-term litter experimental (EBIS) plots soil from the 0 to 10 cm depth interval was collected in 2005 from one plot that had experienced high-^{14}C and one plot with low-^{14}C litter additions at Walker Branch and Haw Ridge sites. For mesocosms, the entire constructed A horizon was used for PLFA analyses. All soil samples were stored in glass jars and frozen immediately after harvest. They were transported from Oak Ridge to Irvine by overnight air in a cooler with frozen gel packs. Once in the laboratory, samples were stored in a freezer at −20 °C until further sample processing.

Prior to extraction of lipids, soil samples were completely defrosted, roots and other fresh plant materials (which contain phospholipids in their cell walls) were removed by sieving to 2 mm and hand picking. Between 200 and 500 g of field-moist soil were extracted by vigorous shaking for 3 h with a mixture of chloroform, methanol and phosphate buffer (1:2:0.8 vol.). After filtration of this mixture and separation of the methanol/water phase from the chloroform phase (which holds the soil lipids), we reduced the volume of the choloroform phase to a few millilitres. This was added to solid phase extraction (SPE) columns filled with silica. Elution was done successively with chloroform, acetone and methanol. Only the methanol fraction, which holds the phospholipids, was collected and again reduced to a few millilitres in volume. Phospholipids were derivatized by mild alkaline methanolysis using a methanolic KOH-solution to obtain fatty acid methyl esters (FAME). We further separated them into saturated, monounsaturated and poly-unsaturated fractions of FAME using aminopropyl-modified and silver nitrate impregnated SPE-columns. A more detailed description of preparation procedures can be found in Bligh and Dyer (1959), Zelles (1999) and Kramer and Gleixner (2006).

Fatty acid fractions were analyzed by gas chromatography/mass spectrometry (GC/MS). Separation was achieved on a DB5 MS fused silica capillary column (30 m × 0.32 mm ID, 0.25 μm film thickness) and identification of present FAME was done by mass spectra, with help of the MS-library and with comparison of mass spectra of FAME in samples and standard materials. The GC-temperature was programmed from 50 °C to 290 °C with a heating rate of 10 °C min^{-1} followed by a heating rate of 20 °C min^{-1} to 320 °C. Data were evaluated with the software X-calibur.

Individual FAME were isolated by preparative capillary gas chromatography (PCGC) using a gas chromatograph coupled to a preparative fraction collector (PFC, Gerstel, Germany). Fatty acid fractions were separated on a CP Sil 8 CB ulti-metal GC-column (50 m × 0.53 mm ID, 0.5 μm film thickness; phase: 5% phenyl 95% dimethylpolysiloxane). About 1% of the sample was transferred to a flame ionization detector (Eglinton et al., 1996; Kramer and Gleixner, 2006; Rethemeyer et al., 2004), and peaks identified by retention time were isolated from the other 99% using the cooling traps of the PFC. We isolated individual compounds from the three FAME-fractions with the following GC-temperature programs: saturated and monounsaturated FAME (60 °C to 190 °C with a heating rate of 15 °C min^{-1}, followed by a heating rate of 2 °C min^{-1} to 230 °C, finally temperature was increased to 320 °C hold for 2 min by heating with 20 °C min^{-1}), polyunsaturated FAME (60 °C to 200 °C with a heating rate of 20 °C min^{-1}, followed by a heating rate of 4 °C min^{-1} to 225 °C, finally temperature was increased to 320 °C hold for 2 min by heating with 20 °C min^{-1}). Repeated sequential injections of the same FAME fraction were performed to isolate sufficient material of individual compounds in the fraction collector traps for analysis of radiocarbon by accelerator mass spectrometry (AMS). Depending on the amount in a FAME fraction, between 10 and 40 injections of each sample were required to isolate 10–50 micrograms of an individual compound.

Purified FAME compounds were transferred from the PFC sample traps to storage vials using dichloromethane and the purity of the isolated compound, as well as the molecular identification were verified by GCMS at the UCI organic mass spectrometry facility

(Greaves, 2002). The sample was then transferred to a precombusted quartz combustion tube, and the solvent was evaporated under a stream of pure N_2. Precombusted cupric oxide and silver wire were added and the tube sealed under vacuum and heated to 900C for 2 hours for combustion of organic C to CO_2. The CO_2 was purified cryogenically and reduced to graphite using standard methods, scaled down for small sample sizes where necessary (Santos et al., 2007). Graphite samples were analyzed at the UCI W.M. Keck Carbon Cycle Accelerator Mass Spectrometry Facility (Southon et al., 2004).

Finally, the FAME radiocarbon data were corrected for the addition of one ^{14}C-free methyl C, which was added during derivatization with methanol/KOH-solution, to calculate the original ^{14}C signature of the PLFA. (Eq. (1))

$$\Delta^{14}C_{FA} = \frac{(N \times \Delta^{14}C_{FAME} - \Delta^{14}C_{MeOH})}{(N-1)} \quad (1)$$

where $\Delta^{14}C_{FA}$ is the isotope value of the fatty acid, $\Delta^{14}C_{FAME}$ the isotope value of the fatty acid methyl ester, $\Delta^{14}C_{MeOH}$ the isotope value of methanol which was used for derivatization and N the number of C atoms in the FAME (Rethemeyer et al., 2004).

2.3.3. Assessment of accuracy and precision of PLFA radiocarbon analyses

Analysis of small (5–50 μg samples) by AMS requires testing with of 'standard' compounds of known-^{14}C put through the same analytical procedures to assess and correct for the addition of small quantities of modern and/or fossil radiocarbon during sample preparation and to assess overall accuracy (Santos et al., 2007). We assessed the amount of 'modern' and 'fossil' (i.e. radiocarbon-free) ^{14}C contamination introduced in the combustion and graphitization procedures by measuring a series of samples of different sizes (spanning 5 μg to 1 mg) derived from a combusted and graphitized oxalic acid standard (modern) or coal (^{14}C-free material). To assess additional contamination associated with the PCGC, we trapped two pure compounds, nC18:0 and nC16:0 FAMEs, with radiocarbon signatures close to those of the PLFA we were analyzing, using multiple injections on the gas chromatograph as for our samples. Because the correction factor for the PCGC varied with time and we were unable to ascertain why, we increased the uncertainty in this correction factor for each sample wheel run until all standards overlapped at the 1-sigma level with the radiocarbon value measured for a 1 mg C sample of the same standard that was not passed through the PCGC (Santos et al., 2007; in press).

2.3.4. Reporting of radiocarbon data: Δ^{14}C notation

We report radiocarbon data as $\Delta^{14}C$, the deviation (in parts per thousand, or‰) of the $^{14}C/^{12}C$ ratio of the sample from that of NBS Oxalic Acid I, the international standard, corrected for radioactive decay of $\Delta^{14}C$ between the year of sampling (200X) and 1950 using the real radiocarbon half-life (Stuiver and Polach, 1977):

$$\Delta^{14}C = \left[\frac{\left[\frac{^{14}C}{^{12}C}\right]_{sample,-25}}{0.95\left[\frac{^{14}C}{^{12}C}\right]_{OX1,-19}\exp^{((200X-1950)/8267)}} - 1 \right] 1000 \quad (2)$$

The sample $^{14}C/^{12}C$ ratio has two corrections: (1) subtraction of backgrounds associated with sample preparation (see above; including correction for the added C during derivatization of PLFA samples) and (2) normalization of $^{14}C/^{12}C$ ratios for mass dependent isotope fractionation by correcting to a common $\delta^{13}C$ of −25‰, assuming ^{14}C is fractionated twice as much as ^{13}C. $\Delta^{14}C$ measurements thus do not reflect mass dependent fractionation, only (in our case) the influence of the ^{14}C added by the 1999 release or subsequent manipulations. The $\Delta^{14}C$ notation corrects for radioactive decay of the standard, but the not sample. In other words, the $\Delta^{14}C$ will be slightly lower if the sample is measured a year later. This difference is ignored in our mass balance calculations, because samples being compared were measured in the same year – in any case over the four years of the experiment, the decline would be <0.5‰.

The precision and accuracy for measurements of graphite targets by AMS are determined by measuring of known-age standards – for standards that are not small samples, these are ±2–3‰ at the WM Keck Carbon Cycle AMS (Xu et al., 2007).

2.3.5. Calculation of leaf litter contribution to microbial C sources/respiration

We calculate F, the fraction of C derived from the labeled leaf litter, by comparing the difference in ^{14}C signature between litter treatment plots and the difference in ^{14}C for the applied litter as in Cisneros Dozal et al. (2006):

$$F = \frac{\left(\Delta^{14}C_{S,high} - \Delta^{14}C_{S,low}\right)}{(970-215)} \quad (3)$$

where $\Delta^{14}C_{S,high}$ and $\Delta^{14}C_{S,low}$ are the radiocarbon signatures for PLFA or respired CO_2 measured in high-^{14}C and low-^{14}C litter treatment plots, respectively; 970‰ and 215‰ are the mean $\Delta^{14}C$ signatures of the added high- and low-^{14}C litter during 2001–2004. We assume that all other possible contributors to microbial C have identical ^{14}C signatures between the experimental plots; that is, any observed difference in ^{14}C between high-^{14}C and low-^{14}C litter treatments is due to incorporation of litter carbon into PLFA or microbially respired CO_2. Prior to litter manipulations, the 1999 release caused an increase in the Δ14C of leaf litter that fell in 2000, which had ^{14}C signatures of 245–260‰ for the Walker Branch Plots and 190–195‰ for the Haw Ridge plots; litter collected for low-^{14}C-enrichment represents a spatial average from this general part of the ORR. In the first year of litter manipulation, the only difference in ^{14}C between plots would have been due to that years' litter addition. In year 2, the difference could have been due to litter added either that year, or the previous year. In year 3, the difference could be from residues from the first or second years, or the final year litter was added. The F value is calculated using the ^{14}C difference in the applied litter, though one could conceivably also calculate the influence of another source (e.g. the Oa horizon carbon) that may dilute that litter C with pre-treatment carbon.

As defined in Eq. (3), F indicates only the influence of leaf litter carbon that is >1 up to 4 years old in the PLFA we sampled from the EBIS plots; for microbially respired CO_2. This ignores the fact that litterfall in 2004 (and prior to 2000) did not differ in its ^{14}C signature between the two plots. We are thus not strictly calculating the only from leaf litter added in the experiment – and therefore could be underestimating the contribution of recent (<1 year) or pre-treatment (>4 years) litter C to PLFA-C. We assess the relative influence of <1 year old litterfall in two ways: (1) by comparing the absolute ^{14}C signatures of the PLFA and most recent (and pre-treatment) litterfall in the EBIS plots; and (2) using the mesocosm experiment, which follows changes in PLFA for 8 months after labeled litter addition.

3. Results

3.1. Reproducibility of PLFA analyses

Radiocarbon data reported for PLFA reflect a number of different errors associated with small sample preparation (combustion and graphitization) and measurement by AMS (Santos et al., 2007). In addition to the corrections for combustion, graphitization and

current-dependent effects in the accelerator mass spectrometer (Santos et al., 2007), we found an additional size dependent deviation associated with the FAME of known-^{14}C signature that were purified in the PCGC. Correction of the known FAME standards assumed that additional ^{14}C-free C was added by the PCGC column separation and trapping procedures. The amount of ^{14}C-free C added by the PCGC varied from near zero to ~2 μg C, though the amount was not predictable from the number of injections. The correction we apply is size dependent and based on Santos et al. (2007), since a constant addition of microgram quantities of C will be a large proportion of the total for very small (several microgram) samples. In addition, we assigned a value of 50% to the uncertainty associated with the amount of 'dead' C from the PCGC (e.g. for 1 μg of added ^{14}C-free C, the uncertainty would be ±0.5 μg). Errors reported here for PCGC data reflect the propagation of all of these uncertainties and are therefore much larger than the precision for larger sample sizes (generally ±3‰ for Modern samples of ~0.5–1 mg C; Southon et al., 2004). Due to the very large errors calculated for samples under 10 micrograms C in size (up to ±120‰), we have excluded them from subsequent analyses. We also excluded samples which when combusted yielded more C than expected based on PLFA peak areas and the number of injections as likely contaminated with residual solvent (see Discussion). Subsequent work in our labs has reduced the errors associated with PLFA analyses (see Druffel et al., in press).

To assess the reproducibility of our PLFA-^{14}C analyses for unknowns, we made two kinds of comparisons (Table 1). First, we report results for an isolated compound separated from the same FAME extract but run through the PCGC at different times (duplicate samples). Second, we report results for the same FAME compound for which we extracted replicate soil samples; these include errors introduced by the analytical procedures associated with extraction, purification and methylation in addition to the PCGC separation and subsequent graphitization (replicate samples). While the quoted errors are large, especially for small samples, the replicate and duplicate analyses fall within the 2-sigma error of the calculated mean for all but one of the compounds (WB3; 18cy; Table 1). Errors were not different between duplicate and replicate samples, and the standard deviation associated with the mean of the replicates was of similar magnitude to the error for each sample. We have used the means and errors as calculated in Table 1 in subsequent analyses of the specific compounds.

Table 1
Radiocarbon signatures (Δ^{14}C in ‰) of phospholipid fatty acids (PLFA) duplicate analyses.

Sample/PLFA	mg C	Δ^{14}C	Err[a]	Mean	Err[b]
I. Separation of the same isolated FAME at different times on the PCGC					
WB3; 16:0 n	0.106	207.7	8.0	205.6	8.7
	0.102	203.6	3.4		
WB4; 16:0 n	0.125	170.2	6.7	194.6	11.9
	0.033	219.0	9.8		
HRH; 16:0 n	0.052	237.9	16.9	246.8	19.0
	0.040	255.5	8.5		
HRL; 18:1	0.171	222.2	2.7	224.3	5.8
	0.067	226.4	5.2		
WB3; 18 cy	0.071	134.3	11.4	189.9	11.9
	0.140	244.7	3.3		
LOA; mar; 15:0 i/a	0.140	135.6	5.7	123.5	24.7
	0.036	111.4	23.9		
LOA; mar; 18:1	0.035	211.0	24.4	184.5	29.3
	0.050	158.0	16.4		
LOA; nov; 18:1	0.198	207.2	4.5	163.9	23.0
	0.035	120.7	22.6		
II. Comparison of the same FAME extracted from replicate soil samples					
LOA18; mar; 18:2	0.029	92.3	45.5	121.1	36.4
LOA42; mar; 18:2	0.035	124.3	39.0		
LOA3A; mar; 18:2	0.027	77.5	49.1		
LOA3B; mar 18:2	0.023	165.7	62.5		
LOA12A, mar; 18:2	0.027	145.4	51.8		
LOA3A; mar; 17:1	0.010	139.3	44.4	141.2	23.0
LOA32A; mar; 17:1	0.025	143.1	17.4		

[a] Error calculated from combining errors associated with dead and modern contributions to small samples analyzed by AMS, including C added to the sample by the PCGC and graphitization (Santos et al. 2007).
[b] Error calculated using propagation of errors when there are two analyses, for the LOA 18:2 multiple extractions, we use the standard deviation of 5 analyses.

3.1.1. Multi-year litter ^{14}C manipulation (EBIS) plots

Fig. 1 summarizes the radiocarbon data for the potential sources of C to microbial PLFA in 2005 in the high-^{14}C and low-^{14}C treatment plots for the Ultisol (Walker Branch) and Entisol (Haw Ridge) sites.

3.1.1.1. Soil organic matter. Radiocarbon signatures of bulk organic matter from 0 to 10 cm depth (roots removed) from which the PLFA samples were extracted ranged from 98‰ (low) to 115‰ (elevated) for Walker Branch litter manipulation plots. Haw Ridge plots had higher bulk soil ^{14}C signatures of 148‰ (low) to 180‰ (elevated). Replicated samples ($n = 4$) of bulk ^{14}C values for the 0–15 cm depth collected in September 2005 had slightly lower ^{14}C values as they integrated C deeper in the soil, but showed a similar pattern (Hanson, unpublished data): 90 ± 4‰ (low) and 92 ± 16‰ (elevated) for Walker Branch and 105 ± 4‰ (low) and 120 ± 15‰ (elevated) for Haw Ridge. Lower ^{14}C values at Walker Branch are in part due to the presence of pre-1963 charcoal, which led to ^{14}C values in low-density fractions separated from the 0 to 15 cm soil of <0‰ (Swanston et al. 2005). We picked charcoal out of the A horizons sampled for our analyses for the Walker Branch site. Charcoal was not in evidence at the Haw Ridge site. O-horizons collected in 2005 had a clearer signal of C incorporated from the 3 years of different litter application: 200 ± 12‰ (low) and 361 ± 25‰ (elevated) at Walker Branch, and 186 ± 10‰ (low) and 308 ± 80‰ (elevated) at Haw Ridge sites (Fig. 1). Radiocarbon signatures of plant material picked out of the soil used for PLFA analysis had ^{14}C signatures similar to those of the O horizon, and likely represent O horizon material that was incorporated into the very large sample. The plant material picked out included leaves and some roots and had Δ^{14}C values ranged from 177–195‰ for low-^{14}C litter plots to 319–384‰ for elevated-^{14}C litter plots. Treseder et al. (2006) demonstrated that there was no difference in Δ^{14}C of roots sampled from the different litter treatment plots. Radiocarbon signatures for living and dead roots in the eastern reserve (sampled outside experimental plots in 2003; Joslin et al., 2006) are also shown in Fig. 1 for comparison. Differences between elevated- and low-^{14}C litter addition plots sampled in August 2005 thus ranged from <20–30‰ in the bulk (plant litter removed) soils from which PLFA were extracted to ~120–190‰ for the overlying O horizon material, from an original difference of ~750‰ between litter treatments (Fig. 1).

3.1.1.2. PLFA. Radiocarbon signatures of PLFA (Table 2; Fig. 1) for Walker Branch and Haw Ridge sites show relatively small differences between compounds or between elevated and low litter label plots. Overall, PLFA averaged 202 ± 19‰ (low-^{14}C) and 236 ± 56‰ (high-^{14}C) for the Haw Ridge sites, not distinguishable from values at the Walker Branch site of 208 ± 39‰ (low-^{14}C) and 215 ± 44‰ (high-^{14}C). With only two exceptions, all PLFA compounds had Δ^{14}C values that were significantly higher than the bulk organic matter from which they were isolated (Table 2 and Fig. 1).

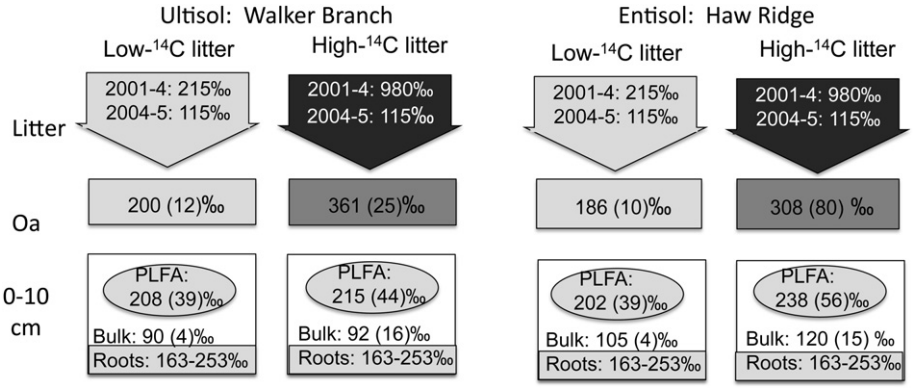

Fig. 1. Comparison of $\Delta^{14}C$ in PLFA (averages over all compounds from Table 2) and potential microbial C sources for the long-term litter manipulation plots (EBIS plots) sampled in 2005. Values given are means, those in parentheses are standard deviations from replicate analyses for soil C and soil fractions; for PLFA they represent the mean (not weighted by compound mass because that would make little difference in this case) across all compounds.

PLFA biomarkers may be used to distinguish among general types of microbes. Saturated PLFA, especially branched compounds, are generally associated with Gram-positive bacteria, while monounsaturated and cyclopropyl-substituted PLFA are associated with Gram-negative bacteria. The PLFA 18:2 ω6 is characteristic for fungi. Averages for each biomarker type were calculated (Table 2) using PLFA 16:0 n, 16:0 br, 17:0 br and 18:0 n for Gram-positive bacteria and 16:1, 17:1, 18:1 and 18 cy for Gram-negative bacteria. Mean values of Gram-negative bacteria and the fungal PLFA marker 18:2 ω6 tend to be greater than Gram-positive PLFA values regardless of treatment (Table 2), though differences are mostly not significant. For the Haw Ridge site, Gram-negative PLFA had higher $\Delta^{14}C$ signatures in the elevated litter plot compared to the lower-^{14}C litter plot.

3.1.1.3. Microbial respiration. Addition of high-^{14}C versus low-^{14}C litter to EBIS Ultisol plots resulted in small but significant increases in the radiocarbon signature of CO_2 in incubation of the 0–5 cm mineral soils (Fig. 2). Similar patterns of small differences in the $\Delta^{14}C$ of CO_2 respired heterotrophically from mineral soils in high-^{14}C versus low-^{14}C litter plots were measured at Walker Branch (eastern reserve) and TVA (western reserve). However, there was a large difference in the ^{14}C signature of 'background' (i.e. independent of litter treatment) radiocarbon signature between the western and eastern sites resulting from the contribution of ^{14}C labeling prior to 2000 (treatments began in 2001), especially to higher labeling of rhizosphere C at the eastern reserve site (TVA; Joslin et al., 2006; Cisneros Dozal et al., 2006; Fig. 2). There is an overall increasing trend in the ^{14}C signature over time for both plots, though in 2003/2004 this is complicated by more ^{14}C releases at the ORR sites. Differences in the ^{14}C signature of heterotrophically respired CO_2 between elevated and low-^{14}C litter treatment plots are larger early in the growing season (May) than late in the growing season (July–September; Fig. 2).

3.1.1.4. Estimates of litter contribution. Values of *F* range from 2 to 8% based on the difference in ^{14}C between CO_2 respired in incubations of soils from Walker Branch EBIS plots during the three years when labeled litter was added to soils (Cisneros Dozal et al., 2006; Fig. 2). Differences in PLFA result in *F* values <12% (Table 2), most are not different from zero given the large analytical errors. Given that the PLFA were collected after a year when leaf

Table 2
Radiocarbon signatures of isolated phospholipid fatty acids (PLFA) from Walker Branch and Haw Ridge. At each site one measured plot is elevated and one plot is low in ^{14}C, $\Delta^{14}C$ values are reported in ‰ with standard deviation. Additionally sample sizes of isolated compounds in mg C are given.

PLFA	WB (elevated)			WB (low)			HR (elevated)			HR (low)		
	mg C	$\Delta^{14}C$	sd	Size	$\Delta^{14}C$	sd	mg C	$\Delta^{14}C$	sd	Size	$\Delta^{14}C$	sd
14:0 n							0.013	159.4	73			
15:0 i	0.015	59.3	26.8							0.031	207.7	10.3
15:0 i/a	0.054	166.5	15.3	0.073	153.8	11.1	0.077	196.4	10.9			
16:0 n		205.6	8.7		194.6	11.9		246.7	19	0.169	214.8	2.8
16:0 br	0.013	190.6	35.7				0.025	236.4	36.3	0.034	204.9	13.3
17:0 n										0.058	214.2	5.6
17:0 br							0.046	194.8	18.7	0.014	183.3	32.8
17:0 br2							0.031	30.2	24	0.01	156.3	43.3
18:0 br										0.035	209.4	12.2
18:0 n				0.023	188.3	38.5	0.056	235.5	15.6	0.026	196.8	16.5
Gram (+) average	**198.1**	**10.6**		**194.6**	**11.9**		**190**	**81**		**197.1**	**21.1**	
16:1 (ω 7;9;11)				0.02	205	16.4	0.076	251.3	4.7	0.018	206.4	18.1
17:01							0.013	271	132	0.08	177.5	2.5
18:1 (ω 9;11;13)	0.06	279	5.8	0.231	238.9	2.3	0.202	264.8	2.8	0.119	224.3	3.9
18:0 cy	0.105	189.5	78.6	0.071	192.7	4.7	0.064	246.8	5.3	0.196	221.7	2.7
Gram (−) average	**234.3**	**63.3**		**212.2**	**23.9**		**258.5**	**11.3**		**207.5**	**21.5**	
18:2ω6	**0.024**	**261.4**	**64.4**	**0.014**	**265.8**	**124.2**	**0.028**	**241.5**	**54.6**	**0.017**	**219.8**	**92.6**

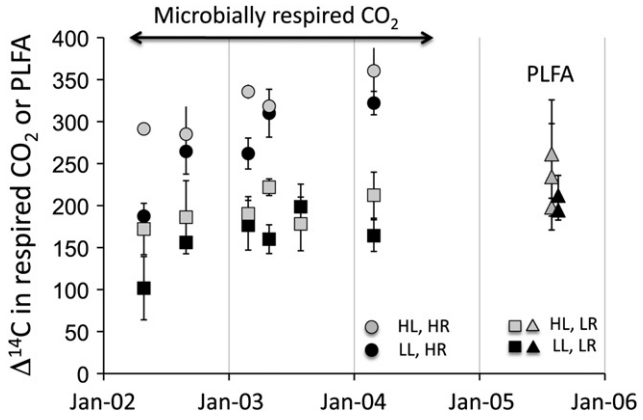

Fig. 2. Radiocarbon signature ($\Delta^{14}C$, *y*-axis) of heterotrophically respired CO_2 from incubation of 0–5 cm mineral soil cores as described in Cisneros Dozal et al. (2006) for Walker Branch (western ORR, Ultisol; squares; LR = low-^{14}C inputs to roots) and TVA (eastern ORR, Ultisol, circles; HR = high-^{14}C inputs to roots). HL = high-^{14}C litter plots; LL = low-^{14}C litter plots; Light gray colors indicate elevated-^{14}C (+950–990‰) litter addition plots and black indicates samples taken from low-^{14}C litter (~210–220‰) addition plots. The first litter addition occurred in February 2002. In the autumn of 2004, natural litterfall with $\Delta^{14}C$ values of 110–120‰ fell at Walker Branch. Averages for PLFA compounds sampled from the WB plots in 2005 (triangles, with averages of Gram-negative and Gram-positive as well as overall average PLFA) have been added for comparison with the time history of microbially respired CO_2 from these plots with the same colors representing high-^{14}C and low-^{14}C litter additions. Some data within the figure are offset in time so that all symbols are distinguishable.

litterfall ^{14}C was not different between the two plots, *F* represents only the contribution of litter that is 1–4 years old. *F* may underestimate overall leaf litter contribution because there was no difference in ^{14}C in for litter that fell in the fall of 2004, prior to our sample collection, or in the years prior to our litter manipulation experiment.

3.1.2. Mesocosm experiment

The radiocarbon signatures of PLFA in the mesocosm A horizons declined from an overall mean across all compounds of 134 ± 33‰ in March (prior to addition of litter), to 120 ± 45‰ in November (Table 3, Figs. 3 and 4). Radiocarbon values in bulk A horizon soil organic matter ^{14}C remained unchanged over this period

Table 3
Radiocarbon signatures of several phospholipid fatty acids isolated from LOA-mesocosm experiments at the beginning of the experiment and after 8 months. Data are reported as $\Delta^{14}C$ in ‰ with standard deviation sd. Also sample sizes in mg C are shown; an asterisk indicates the number is an average of multiple analyses (see Table 1).

PLFA	LOA-mesocosm (initial)			LOA-mesocosm (8 months)		
	Mar-06			Nov-06		
	mg C	$\Delta^{14}C$	sd	mg G	$\Delta^{14}C$	sd
14:0 n	0.015	91.9	56.7			
15:0 i/a	*	123.5	24.7	0.098	144.4	8
16:0 n	0.043	155.6	23.5	0.113	139.7	7.1
16:0 br	0.06	119	12.9	0.031	100.9	28.6
17:0 br	0.063	130.7	12.6	0.05	68.8	15
17:0 br2	0.077	118.9	10.1	0.042	81.1	18.3
18:0 n	0.098	135.3	8.2	0.067	94.9	11.7
Gram (+) average		**131.9**	**15.1**		**97.1**	**26.9**
16:1 (ω 7;9;11)	0.1	151.8	8.1	0.056	127	14
17:1	*	141.2	47.7			
18:1 (ω 9;11;13)	*	184.5	37.5	*	163.9	61.1
Gram (−) average		**159.2**	**22.6**		**145.5**	**26.1**
18:2ω6	*	**121.6**	**36.4**			

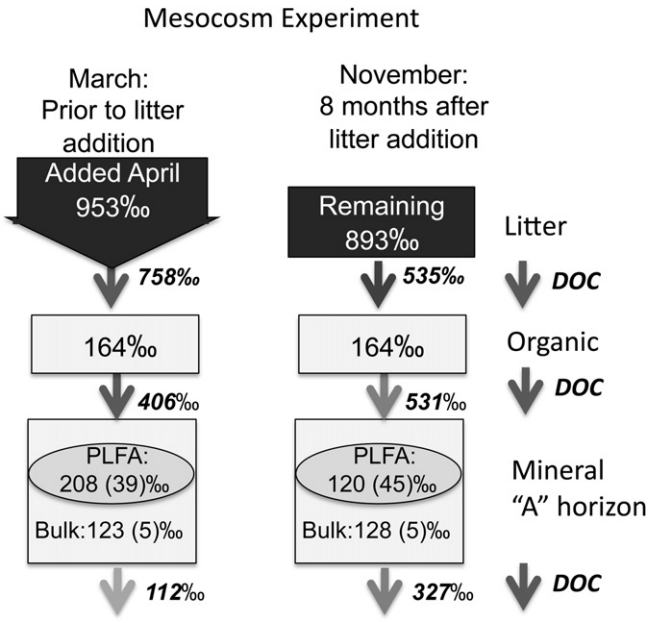

Fig. 3. Radiocarbon ($\Delta^{14}C$) in PLFA compounds (averages from Table 3 and Fig. 4) and potential C sources compared experimental mesocosms in March (prior to litter addition) versus November (~8 months after litter addition) in 2006. Numbers associated with arrows are the $\Delta^{14}C$ signatures of DOC transferred between levels of the mesocosms in April (i.e. Just after litter addition) and November; data are from Fröberg et al. (2009).

(+123 ± 4‰; Fröberg et al., 2009). Decreases in ^{14}C from March to November were significant for some of the Gram-positive bacterial PLFA (e.g. 17:0 branched compounds), with some decreasing to below the bulk ^{14}C soil organic matter value (Fig. 4). Gram-negative bacteria tended to have higher $\Delta^{14}C$ values compared to the average, and declined less over the 8-month period.

4. Discussion

4.1. Multi-year influence of the litter experiments on mineral soil microbial C sources

Both measures of microbial C source ^{14}C signatures – microbial respiration and individual PLFA biomarkers – show either small or no differences between high- and low-^{14}C litter addition plots. Results were the similar whether the measurement was made months (heterotrophic respiration and mesocosm experiment)

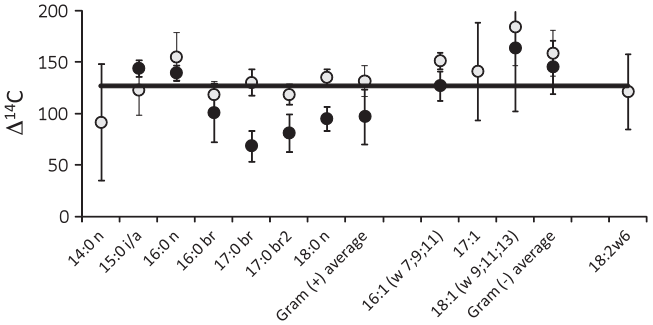

Fig. 4. Radiocarbon ($\Delta^{14}C$) in single PLFA compounds compared for samples extracted from the A horizon of experimental mesocosms (see text) in March (gray circles, prior to litter addition) versus November (black circles, ~8 months after start) in 2006. The bulk organic matter $\Delta^{14}C$ signature, which did not change with time, is given as the solid line.

or nearly 4 years (PLFA from long-term experimental plots) following litter addition onset (Fig. 2).

Our method for calculating F means it is only assessing the contribution of C from the years when ^{14}C-labeled litter was added to the plots (i.e. 1–4 year old C). It is thus possible that the EBIS plot PLFA results underestimate the overall contribution from leaf litter C because there was no difference in ^{14}C for leaf litter that is <1 year old or >4 years old, and this could be diluting the difference between plots. While we cannot quantitatively assess this effect, we can assess whether or not the majority of C can be coming from leaf litter sources outside of our manipulation by comparing absolute ^{14}C signatures. The ^{14}C signature of the PLFA sampled in 2005 (Fig. 1) average 200–220‰, while the litter that fell in autumn of 2004 had $\Delta^{14}C$ of 110–120‰ in both plots. Hence the lack of difference in PLFA between plots cannot be exclusively due to incorporation of <1 year leaf litter carbon; indeed if current year leaf litter were a major source of PLFA-C, we would need to find another source of PLFA-C that had much higher ^{14}C signatures than we measure in the other soil components (see Fig. 1). The same is true for C derived from leaf litter that pre-dates the experimental treatment. While the $\Delta^{14}C$ of leaf litter in 2000 was 245–260‰ at Walker Branch and 109–195‰ at Haw Ridge (leaf litter in this part of the ORR was the source for the low-^{14}C litter addition), the $\Delta^{14}C$ of leaf litter that fell in prior years was much lower, closer to 100‰ (Hanson et al., 2005). The lack of a strong signal from recent leaf litter in microbial respiration (in years when <1 year old leaf litter had very different ^{14}C signatures), and the lack of a litter-derived ^{14}C signature in the mesocosms in the 8 months following litter addition, indicate that a major contribution from <1 year litter is unlikely. While it is conceivable that the lack of difference in PLFA-^{14}C could be derived 100% from the 2000 litterfall, there could be minimal contributions from the years before or after, which we find improbable.

Microbial respiration and PLFA data also demonstrate that microbial C sources have higher ^{14}C signatures than bulk organic C in the mineral soil, indicating that soil organic matter is likely not the dominant C source (Figs. 1, 3). The radiocarbon signatures of the Oa horizons are closer to those of microbial C sources. However, given the 120–160‰ difference in $\Delta^{14}C$ of the Oa horizon carbon between high-^{14}C and low-^{14}C litter treatment plots (Fig. 1), and the <40‰ difference in PLFA-^{14}C, the maximum contribution of Oa horizon C to microbes in 2005 would be <33% (i.e. F for Oa source C < 40/120). The overall increase in the ^{14}C signature of microbially respired CO_2 with time (Fig. 2) is not related to the addition of elevated-^{14}C litter, since it occurs in both elevated- and low-^{14}C litter treatments to the same degree.

In both treatment plots, the ^{14}C signature of fine roots reported by Joslin et al. (2006) is closest to the ^{14}C signatures recorded in the PLFA compounds, suggesting that roots are the major source of C in the mineral soil microbial community (Fig. 3).

4.2. Mesocosm experiment

The mesocosm experiment confirmed that C from added litter is not significantly incorporated into microbial PLFA. Depending on the compound isolated, there is either no difference in the ^{14}C of PLFA over the course of 8 months, or there is a decline of up to 40–60‰ (Figs. 3 and 4). This decline tends to be largest for unsaturated compounds typical of Gram-negative bacteria (Fig. 4). Since the decline takes place in spite of the fact that elevated-^{14}C litter was added to the surface of the mesocosm just after the first sampling (March), it indicates the loss of a C source with higher ^{14}C values during the experiment. Carbon contents in the mesocosm A horizons declined during the experiment (Fröberg et al., 2009), though there was no measurable change in the radiocarbon signature of bulk A horizon organic matter. The radiocarbon content of DOC draining litter and organic layers and entering the A horizon was 406‰ in April (after labeled litter addition and following our March sampling) and 531‰ in November (our second sampling; Fig. 3). DOC draining the A horizon increased in ^{14}C content from 112 to 327‰ over the same time period (Fröberg et al., 2009; Fig. 2). While DOC carbon is sequestered in the mesocosm A horizons during this time period (Fröberg et al., 2009), it is not primarily derived from recent leaf litter given the decline or lack of change in ^{14}C in microbial biomarkers. Given that root inputs were excluded in the mesocosm experiment, the decline in C content in the A horizon, and in radiocarbon signature of PLFA, tend to corroborate the idea that roots, rather than Oa horizon carbon, are the major source of C for microbes. As in the Walker Branch and Haw Ridge sites, Gram-negative bacteria PLFA show the largest change with time; it is clear in this instance that the C source is not leaf litter. If DOC from the Oa horizon were a major source, we should have seen an increase in $\Delta^{14}C$ of PLFA, rather than the observed decline (Fig. 2).

4.2.1. Issues with compound specific analysis

One goal of this investigation was to test whether PLFA biomarker isotopes agreed with the more general measures of microbial C sources. While unfortunately we have no direct comparison of PLFA with respired CO_2, the overall conclusion – that C sources from surface leaf litter are small and that the major sources are C derived from roots rather than older soil C sources – is common to both measures. Differences between the radiocarbon signatures of specific PLFA biomarkers potentially add insights to the how C sources may differ for general types of bacteria and fungi. However, the differences between individual biomarkers measured in this study were small and mostly not significant. Given the large errors associated with the measured radiocarbon signatures for many of the PLFA compounds due to small sample size and uncertainty in background corrections, and the fact that we were attempting to detect a very large label signature, it is clear that great care must be taken in interpreting PLFA radiocarbon data from natural ^{14}C samples.

We have greater temporal resolution in sampling of microbial respiration than for PLFA (Fig. 2). There is some indication from the microbial respiration data that a larger contribution of C (up to 8–12%) occurs in spring (Fig. 3 and Cisneros Dozal et al., 2006) compared to later in the growing season. Cores for heterotrophic respiration were sampled a minimum of 3 and a maximum of 10 months following the addition of fresh litter to the soil in November–February. We cannot rule out that measurements made within a few days to weeks of the litter addition would yield higher values of *F*, if there is greater export of ^{14}C-DOC from the freshly applied litter early in the season, or changes in the source of C to the microbial community over the course of the season.

There are two PLFA compounds with ^{14}C values less than bulk soil C and for which we do not have a good explanation (15:0i in the elevated-^{14}C litter plot of Walker Branch and 17:0br2 in the elevated-^{14}C litter plot of in Haw Ridge; Table 2). We experienced some trouble in developing our PLFA analysis with the incomplete evaporation of solvents used to transfer the purified compounds. This clearly occurred when the CO_2 yield on combustion of the sample greatly exceeded the amount expected based on the peak size detected by the gas chromatograph. These samples (normally very small ones, e.g. <30 μg C) always had very low-^{14}C values and have been excluded from the data set. In these two cases we did not see clear excesses of yield. However, a ~50‰ decrease in the radiocarbon signature could be caused by ~5% of the combusted C being radiocarbon-free solvent; given the uncertainties in the calibration of the gas chromatograph and in our measurement of

evolved CO_2, such a discrepancy would be difficult to detect. We do suspect that systematic errors in PLFA tend to yield values that are lower in ^{14}C rather than higher, since the contaminants we have identified as originating from solvents or from the PLFA separation procedures (e.g. column bleed) both add ^{14}C-free C to PLFA with modern ^{14}C values. We are unable to assess the potential for modern C contamination by our procedures because we could not find a radiocarbon-free FAME standard. Single compound measurements of radiocarbon require careful attention to detail and it is clear that accurate measurement of very small samples requires assessment of the effects of all steps in the analysis on the result. We have thus chosen to report data with large analytical errors; duplicate and replicate analyses indicate that these large errors are appropriate (Table 1). Subsequent to the work performed for this paper, we have made a number of improvements to reduce the error, most notably by replacing the gas chromatography column (see Druffel et al., in press, for details).

5. Conclusions

Measures of two proxies for microbial C sources in mineral soil both show that <10% of the C in or respired by microbes is derived from surface leaf litter that had been manipulated in ^{14}C content; the manipulations took place either 1–4 years (EBIS plots) or <1 year (mesocosms) ago. While we cannot completely rule out the possibility that our EBIS plot results underestimate the contribution of C from leaf litter that fell before (>4 years) or after (<1 years) the experimental period, three additional lines of evidence make a major contribution from such a source unlikely. The first is the correspondence of PLFA-^{14}C values with those of roots sampled at the same time, but not with the most recent or pre-treatment litterfall. The second is the small contribution of leaf litter C to the CO_2 respired heterotrophically in the mineral soil throughout the period when litter manipulations occurred. The third is the lack of measured influence of <1 year leaf litter in the mesocosm PLFA experiment.

Although the ^{14}C associated with labeled litter additions resulted in a cumulative difference in the ^{14}C of Oa horizons over three years, there was no apparent accumulation of litter-derived C products in soil A horizon microbes over time.

PLFA single compound measurements suggest that more of the surface litter C was incorporated by Gram-negative bacteria and fungi than Gram-positive bacteria. However, analytical uncertainties associated with PLFA measurements limit these interpretations.

The mean ^{14}C signature of PLFA compounds was higher than that for the bulk soil organic matter in both of our experiments, indicating that SOM is not the major source for microbial C. While ^{14}C signatures of PLFA were between those of bulk organic matter from the Oa and A horizons, the lack of a difference in PLFA-^{14}C between high- and low-^{14}C treatments in our litter addition experiment limits contribution form the Oa horizon to be <33% of microbial C. Further, the lack of apparent uptake of DO^{14}C draining the O horizon in our microcosms, suggest that the Oa horizon is not a major source of C to microbes. We conclude that roots, which have overall ^{14}C signatures closest to those we observed for PLFA are the major source of C for microbes in the A horizon at our sites.

Single compound radiocarbon measurements, particularly of very small (<30 μg C) samples, have large associated uncertainties because of contamination of the sample when processed by the PCGC. All single compound radiocarbon data should be accompanied by assessments of overall accuracy and precision, including processing of known-^{14}C sample materials through all stages of sample preparation and handling, and duplicate and replicate analyses.

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References

Bird, J.A., Kleber, M., Torn, M.S., 2008. C-13 and N-15 stabilization dynamics in soil organic matter fractions during needle and fine root decomposition. Organic Geochemistry 39, 465–477.
Bird, J.A., Torn, M.S., 2006. Fine roots vs. needles: a comparison of C-13 and N-15 dynamics in a ponderosa pine forest soil. Biogeochemistry 79, 361–382.
Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 37, 911–917.
Brant, J.B., Myrold, D.D., Sulzman, E.W., 2006a. Root controls on soil microbial community structure in forest soils. Oecologia 148, 650–659.
Brant, J.B., Sulzman, E.W., Myrold, D.D., 2006b. Microbial community utilization of added carbon substrates in response to long-term carbon input manipulation. Soil Biology & Biochemistry 38, 2219–2232.
Cisneros Dozal, L.M., Trumbore, S., Hanson, P.J., 2006. Partitioning sources of soil-respired CO_2 and their seasonal variation using a unique radiocarbon tracer. Global Change Biology 12, 194–204.
Denef, K., Roobroeck, D., Wadu, M.C.W.M., Lootens, P., Boeckx, P., 2009. Microbial community composition and rhizodeposit-carbon assimilation in differently managed temperate grassland soils. Soil Biology & Biochemistry 41, 144–153.
Druffel, E.R.M., Zhang, D., Xu, X., Ziolkowski, L., Southon, J.R., Santos, G.M., Trumbore, S.E. Compound specific radiocarbon analyses of phospholipid fatty acids and n-alkanes in ocean sediments. Radiocarbon, in press.
Eglinton, T.I., Aluwihare, L.I., Bauer, J.E., Druffel, E.R.M., McNichol, A.P., 1996. Gas chromatographic isolation of individual compounds from complex matrices for radiocarbon dating. Analytical Chemistry 68, 904–912.
Fröberg, M., Berggren, D., Bergkvist, B., Bryant, C., Knicker, H., 2003. Contributions of oi, oe and oa horizons to dissolved organic matter in forest floor leachates. Geoderma 113, 311–322.
Fröberg, M., Hanson, P.J., Trumbore, S.E., Swanston, C.W., Todd, D.E., 2009. Flux of carbon from C-14-enriched leaf litter throughout a forest soil mesocosm. Geoderma 149, 181–188.
Fröberg, M., Kleja, D.B., Hagedorn, F., 2007. The contribution of fresh litter to dissolved organic carbon leached from a coniferous forest floor. European Journal of Soil Science 58, 108–114.
Gaudinski, J.B., Torn, M.S., Riley, W.J., Swanston, C., Trumbore, S.E., Joslin, J.D., Majdi, H., Dawson, T.E., Hanson, P.J., 2009. Use of stored carbon reserves in growth of temperate tree roots and leaf buds: analyses using radiocarbon measurements and modeling. Global Change Biology 15, 992–1014.
Greaves, J., 2002. Operation of an academic open access mass spectrometry facility with particular reference to the analysis of synthetic compounds. Journal of Mass Spectrometry 37, 777–785.
Hanson, P.J., Swanston, C.W., Garten, C.T., Todd, D.E., Trumbore, S.E., 2005. Reconciling change in oi-horizon carbon-14 with mass loss for an oak forest. Soil Science Society of America Journal 69, 1492–1502.
Joslin, J.D., Gaudinski, J.B., Torn, M.S., Riley, W.J., Hanson, P.J., 2006. Fine-root turnover patterns and their relationship to root diameter and soil depth in a C-14-labeled hardwood forest (172, pg 523, 2006). New Phytologist 173, 523–535.
Kramer, C., Gleixner, G., 2006. Variable use of plant- and soil-derived carbon by microorganisms in agricultural soils. Soil Biology & Biochemistry 38, 3267–3278.
Kramer, C., Gleixner, G., 2008. Soil organic matter in soil depth profiles: distinct carbon preferences of microbial groups during carbon transformation. Soil Biology & Biochemistry 40, 425–433.
Leckie, S.E., 2005. Methods of microbial community profiling and their application to forest soils. Forest Ecology and Management 220, 88–106.
Moore-Kucera, J., Dick, R.P., 2008. Application of C-13-labeled litter and root materials for in situ decomposition studies using phospholipid fatty acids. Soil Biology & Biochemistry 40, 2485–2493.
Rethemeyer, J., Kramer, C., Gleixner, G., Wiesenberg, G.L.B., Schwark, L., Andersen, N., Nadeau, M.J., Grootes, P.M., 2004. Complexity of soil organic matter: AMS C-14 analysis of soil lipid fractions and individual compounds. Radiocarbon 46, 465–473.

Riley, W.J., Gaudinski, J.B., Torn, M.S., Joslin, J.D., Hanson, P.J., 2009. Fine-root mortality rates in a temperate forest: estimates using radiocarbon data and numerical modeling. New Phytologist 184, 387–398.

Rubino, M., Lubritto, C., D'Onofrio, A., Terrasi, F., Kramer, C., Gleixner, G., Cotrufo, M. F., 2009. Isotopic evidences for microbiologically mediated and direct C input to soil compounds from three different leaf litters during their decomposition. Environmental Chemistry Letters 7, 85–95.

Santos, G.M., Moore, R.B., Southon, J.R., Griffin, S., Hinger, E., Zhang, D., 2007. AMS C-14 sample preparation at the KCCAMS/UCI facility: status report and performance of small samples. Radiocarbon 49, 255–269.

Santos, G.M., Southon, J.R., Drenzek, N.J., Ziolkowski, L.A., Druffel, E.R.M., Xu, X., Zhang, D., Trumbore, S., Eglinton, T.I., Hughen, K. Blank assessment for ultra-small radiocarbon samples: chemical extraction and separation vs. AMS. Radiocarbon, in press.

Southon, J., Santos, G., Druffel-Rodriguez, K., Druffel, E., Trumbore, S., Xu, X.M., Griffin, S., Ali, S., Mazon, M., 2004. The keck carbon cycle AMS laboratory, university of california, irvine: initial operation and a background surprise. Radiocarbon 46, 41–49.

Stuiver, M., Polach, H., 1977. Reporting of C-14 data. Radiocarbon 19, 355–363.

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

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

Trumbore, S., Gaudinski, J.B., Southon, J.R., Hanson, P.J., 2002. Quantifying ecosystem-atmosphere carbon exchange with a ^{14}C label. EOS. Transactions of the American Geophysical Union 84, 265–268.

Waldrop, M.P., Balser, T.C., Firestone, M.K., 2000. Linking microbial community composition to function in a tropical soil. Soil Biology & Biochemistry 32, 1837–1846.

Waldrop, M.P., Firestone, M.K., 2006. Seasonal dynamics of microbial community composition and function in oak canopy and open grassland soils. Microbial Ecology 52, 470–479.

Xu, X.M., Trumbore, S.E., Zheng, S.H., Southon, J.R., McDuffee, K.E., Luttgen, M., Liu, J.C., 2007. Modifying a sealed tube zinc reduction method for preparation of AMS graphite targets: reducing background and attaining high precision. Nuclear Instruments & Methods in Physics Research Section B – Beam Interactions with Materials and Atoms 259, 320–329.

Zelles, L., 1999. Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: a review. Biology & Fertility of Soils 29, 111–129.