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Fine roots vs. needles: a comparison of ^{13}C and ^{15}N dynamics in a ponderosa pine forest soil

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Abstract. Plant allocation patterns may affect soil C and N storage due to differences in litter quality and the depth of plant C and N inputs into the soil. We studied the dynamics of dual-labeled ($^{13}C/^{15}N$) *Pinus ponderosa* needles and fine roots placed at two soil depths (O and A horizon) in a temperate conifer forest soil during 2 y. Input of C as fine roots resulted in much more C retained in soil (70.5 ± 2.2% of applied) compared with needle C (42.9 ± 1.3% of applied) after 1.5 y. Needles showed faster mass loss, rates of soil $^{13}CO_2$ efflux, and more ^{15}N immobilized into microbial biomass than did fine roots. The larger proportion of labile C compounds initially present in needles (17% more needle C was water soluble than in fine roots) likely contributed to its shorter C residence time and greater degree of transformation in the soil. A double exponential decay function best described the rate of ^{13}C loss, with a smaller initial pulse of C loss from fine roots (S_1k_1) and a slower decay rate of the recalcitrant C pool for fine roots (0.03 y^{-1}) compared with (0.19 y^{-1}) for needles. Soil ^{13}C respiration, representing heterotrophic respiration of litter C, was much more seasonal from the O horizon than from the A. However, offsetting seasonal patterns in ^{13}C dynamics in the O horizon resulted in no net effect of soil depth on total ^{13}C retention in the soil after 1.5 y for either litter. Almost 90% of applied litter N was retained in the soil after 1.5 y, independent of litter quality or soil depth. Very small amounts of ^{13}C or ^{15}N (< 3% of applied) moved to the horizon above or below the placement depth (i.e., O to A or A to O). Our results suggest that plant allocation belowground to fine roots results in more C retained and less N mineralized compared with allocation aboveground to needles, primarily due to litter quality differences.

Abbreviations: CFE – chloroform fumigation extraction; DED – double exponential decay; IRMS – isotope ratio mass spectrometer; SED – single exponential decay; SOM – soil organic matter

Introduction

Many of the important factors regulating the decomposition rates of aboveground litter are known for forest ecosystems (Melillo et al. 1982; Aber et al. 1990; Aerts 1997; Kurz et al. 2000). However, for fine roots, the controls on

decay, the proportion of C retained in soil, and the mineralization dynamics of N are less well understood (Zak and Pregitzer 1998; Silver and Miya 2001). While fine roots are generally a smaller proportion of forest ecosystem biomass than coarse roots (Vogt et al. 1987), they are much more dynamic (Raich and Nadelhoffer 1989) and have been estimated to contribute 33% of global annual net primary production (Jackson et al. 1997). The turnover of foliar litter and fine roots are the main sources of soil C (Dixon et al. 1994) and also key drivers of biogeochemical cycling in forest ecosystems, especially soil N (McClaugherty et al. 1982; Vogt et al. 1986; Chen et al. 2002). Consequently, a better understanding of the dynamics of fine root C and N in soil is needed to assess the productivity of temperate forest ecosystems and to represent the effects of plant allocation patterns in ecosystem models.

Much of our current knowledge of foliar litter and fine root decomposition rates and their control has been gained from the buried litterbag technique (Aerts 1997; Silver and Miya 2001). Litterbags are an efficient method of quantifying and comparing the decay rates of many substrates and environments. Fine root decay measured with litterbags, however, appears low compared with other *in situ* methods (Fahey et al. 1988; Hendrick and Pregitzer 1992; Fahey and Arthur 1994). The litterbag method may limit litter contact with the soil and decomposers, and thus retard mass loss and nutrient release. For example, Dornbush et al. (2002) found 10–23% lower mass loss and 21–29% lower N release from fine roots in litterbags compared with intact cores. More significantly, however, the litterbag, tethered root, minirhizotron, and intact core methods provide only the mass loss rate (i.e., disappearance of intact litter) and thus do not quantify the amount of litter C or N retained in the soil. Understanding the nature and quantity of C and N products stabilized in soil as litter decays (i.e., dissolved C and N, microbial C and N, and humic substances) is critical to advancing our knowledge of decomposition and stabilization processes and to providing insight into potential long-term C and N storage. In this investigation, we used a dual-labeled ($^{13}C/^{15}N$) litter approach intended to achieve a more complete inventory of C and N dynamics *in situ* and minimize methodological artifacts.

Soil horizons can differ in many ways that are potentially important to plant litter decomposition and soil C and N stabilization, including pedoclimate, nutrient availability, soil texture, metal oxide concentrations, and microbial community composition. In temperate forests, typically 50% or more of fine roots occur at a soil depth of less than 40 cm (Harris et al. 1977; Joslin and Henderson 1987; Hendrick and Pregitzer 1996). The depth of belowground C and N deposition may be affected by forest management (e.g., tree species planted, understory removal), disturbance (e.g., fire), or the potential effects of global climate change (e.g., rooting patterns or the proportion of C allocated to above- and belowground plant components) (Norby and Jackson 2000). Decomposition rates for fresh roots declined linearly with increasing soil depth from 10 to 100 cm (Gill and Burke 2002). In contrast, more plant C and N was retained in temperate forest soils near the soil surface compared to deeper

depths (Rovira and Vallejo 1997, 2002). Accordingly, there is a need to better quantify how the depth of plant litter inputs in the soil affects C and N retention.

This research addressed two main questions: (1) Does plant allocation belowground (fine roots) vs. aboveground (needles) in temperate forests increase C and N retention in soils? (2) How does the depth of plant inputs in the soil (O vs. A horizon) affect the retention of C and N from fine root and needles? A field study was initiated in 2001 to follow the fate of dual-labeled (^{13}C and ^{15}N) *Pinus ponderosa* fine roots (< 2 mm) and needles.

Materials and methods

Study site

The study site is located at 1315 m a.s.l. in the Blodgett Experimental Forest, on the western slope of the Sierra Nevada in El Dorado County, CA, USA (120°38′30″ W; 38°53′00″ N). The soil is classified as a sandy, mixed, mesic Ultic Haploxeralf and is derived from granite (Soil Survey Staff 1999). The vegetation is 90-year-old conifer forest dominated by ponderosa pine (*Pinus ponderosa*), white fir (*Abies concolor*), and incense cedar (*Calocedrus decurrens*). Shrubs are sparse under the 30–45 m tall canopy and include *Arctostaphylos* spp. and *Ceanothus* spp. A well-developed organic O horizon is present at the site and contained similar quantities of C as the top 10 cm of the A horizon (Table 1). The climate is Mediterranean with warm, dry summers and cool, wet winters. Annual precipitation averaged 1774 mm (1962–2001) and is concentrated between November and April (88%), with much falling as snow.

Soil volumetric moisture content and soil temperature were monitored at 15 min intervals for 2 depths (i.e., 3 cm below surface and 3 cm below the O/A horizon interface) at four locations within the study site. Soil moisture content measurements were made using 30 cm long water content reflectrometers (C615, Campbell Scientific, Logan UT, USA) inserted parallel to the soil surface. Soil temperature measurements were made using Type T thermocouples. In the O horizon, the soil was cooler and wetter during the winter and spring, and warmer and drier in the summer and autumn than in the A horizon, with greater temporal variability (Figure 1). Soil moisture and temperature had a strong negative correlation in both the O horizon ($r = -0.824$) and the A horizon ($r = -0.887$).

^{13}C and ^{15}N plant litter

Two-year-old ponderosa pine saplings were grown and labeled with $^{13}CO_2$ and $^{15}NO_3^-$ under controlled greenhouse conditions to produce a season's growth of uniformly labeled needles and fine roots. A 50% soil (from the study site)

Table 1. Soil C, N, ^{13}C, ^{14}C, ^{15}N, C turnover time, bulk density, and horizon thickness of soil size fractions from the O and A horizon (0–10 cm depth).

Soil horizon	Size fraction	Total C (g kg^{-1})	Total N (g kg^{-1})	$\delta^{13}C$ (‰)	$\Delta^{14}C$ (‰)	C turnover time (y^{-1})	C:N	Bulk density (g cm^3)	Hortizon thickness (cm)
O	> 2 mm	438 (10)	9.2 (0.5)	−27.2 (0.1)	168	9	48 (1)		
O	< 2 mm	268 (23)	8.2 (0.2)	−26.7 (0.1)	214	20	32 (1)		
O	Total	338 (20)	8.5 (0.4)	−26.9 (0.1)			39 (1)	0.13 (0.01)	8.2 (0.4)
A	> 2 mm	331 (11)	6.1 (0.4)	−26.4 (0.2)	155	7	61 (8)		
A	< 2 mm	48 (2)	1.9 (0.1)	−25.6 (0.1)	102	85	26 (1)		
A	Total	57 (6)	1.9 (0.1)	−25.7 (0.1)			29 (1)	0.84 (0.03)	10.0

Soil sampled in Apr. 2002 (n = 16), except natural abundance isotope enrichment sampled in Nov. 2001 (n = 4). Standard errors of means shown in parentheses.

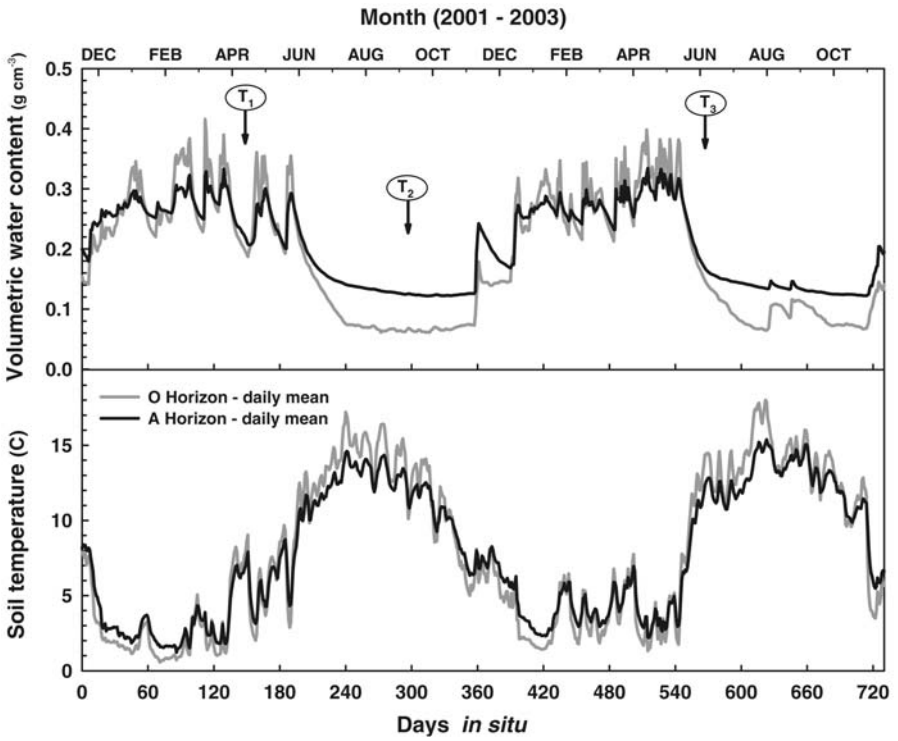

Figure 1. Daily mean soil moisture (top) and soil temperature (bottom) for the O horizon (2–4 cm depth; grey lines) and the A horizon (2–4 cm below O/A horizon interface; black lines) during the 2 y after application of the ^{13}C-labeled substrates. Daily mean values represent averages of measurements from four field replicates made every 15 min. Arrows indicate dates when soil excavations were made.

and 50% fritted clay medium was used to provide support and to facilitate root recovery. Fertilizer ^{15}N as KNO_3 was added weekly at 30% atom excess. Labeling was accomplished in a climate-controlled, plexiglass growth chamber modified from Bird et al. (2003). Pine saplings were exposed to one photoperiod of enriched $^{13}CO_2$ (10% atom percent) 10 times during the course of their development (i.e., ca. every 7–10 d after bud break) to maximize the uniformity of ^{13}C enrichment. After maximum needle elongation, water was withheld from the samplings prior to harvest of fine roots and needles to prompt senescence. Needles and fine roots produced during the ^{13}C-labeling season were harvested for field application by clipping fine roots and needle clusters. Fine roots and needles were dried at 25 °C, and homogenized prior to addition to the field microcosms. Labeled fine roots were < 2 mm in diameter and at least 7.6 cm long. The ^{13}C enrichment of needles (2487‰) exceeded fine roots (2069‰). The ^{15}N enrichment of fine roots (8.1 atom%) exceeded needles (5.5 atom%). Total litter C and N were determined using a CHN gas analyzer (Nelson and Sommers 1982; Table 2).

Table 2. C and N composition of *Pinus ponderosa* needles and fine roots added to soil in November 2001.

Litter type	Total		Proximate C fraction							Ratios	
	C ($g\ kg^{-1}$)	N ($g\ kg^{-1}$)	NPE ($g\ kg^{-1}$)	WS ($g\ kg^{-1}$)	WS phenol ($g\ kg^{-1}$)	WS gulcose ($g\ kg^{-1}$)	AHF ($g\ kg^{-1}$)	AHF gulcose ($g\ kg^{-1}$)	ARF ($g\ kg^{-1}$)	C:N	ARF:N
Needles	485	12.4	42.8	378	27.5	129.1	369	195	210	38.9	16.9
Fine roots	463	9.5	46.7	211	15.7	44.9	469	263	274	48.6	28.8

Mean values of three subsamples are shown.

All proximate C values are expressed on an ash-free dry matter basis. NPE, non-polar extractives; WS, water soluble extractives; WS phenol, water soluble phenol expressed as percent tannic acid equivalents; WS glucose, water-soluble polysaccharide expressed as percent glucose equivalent; AHF, acid hydrolyzable fraction; AHF glucose, acid hydrolyzable polysaccharides expressed as percent glucose equivalents; ARF, acid resistant fraction. The sum of NPE, WS, AHF, and ARF fractions equals total C content.

The chemical composition of fine roots and needles was determined on three subsamples of the labeled litter using a forest products determination of proximate C fractions (Ryan et al. 1990; Table 2). Needles had lower C:N and acid-resistant fraction (ARF):N ratios than fine roots (Table 2). While needles and fine roots had similar proportions of non-polar extractable C compounds (4.5%), needles had a higher proportion of water-soluble C compounds and a lower proportion of acid-hydrolyzable C and acid-resistant C compounds (Table 2).

^{13}C- and ^{15}N-litter field study

The experiment was a 2×2 factorial design with four field replications. Factor 1 compared the C and N dynamics of added fine roots vs. needles; Factor 2 compared the effects of soil depth (i.e., 2–4 cm below the O horizon surface vs. 2–4 cm below the O/A horizon interface). Microcosms (10.2 cm diameter, 23 cm long schedule 40 PVC) were inserted into the soil in August 2001 and allowed to equilibrate for 120 d prior to the application of $^{13}C/^{15}N$-labeled litter. Three microcosms per treatment were installed for harvest 152 d (5 mos.), 294 d (10 mos.) and 568 d (1.5 y) after the application of the labeled-litter substrates. Microcosms had two 5 cm diameter windows fit with 450 μm mesh to allow fungal hyphae and fine roots to penetration the core. Microcosms were placed >1 m from large trees and >0.5 m from adjacent microcosms.

On November 16, 2001, $^{13}C/^{15}N$-labeled fine roots or needles were applied to either the O or A horizon of microcosms. Labeled litter was added to soil at a rate of 147 g m^2 (dry matter) for needles and 135 g m^2 for fine roots. Litter was placed in the microcosms and lightly mixed with soil in its placement depth. Regardless of soil placement depth, all microcosms that received litter had similar soil disturbance in both horizons. Additional microcosms served as controls and were either treated like those that received litter (i.e., minimally disturbed soil without litter) or left undisturbed. Total soil CO_2 respiration rates were measured on eight occasions from 2001 to 2003 to assess the disturbance to the soil during litter addition and the effect of added litter. In addition, microbial biomass C and N and extractable inorganic N was measured in April 2002, 5 mos. after disturbance (data not shown), for both control plots. No effects of the initial soil disturbance or the litter addition were observed in any of these measured parameters.

Soil chemical and microbiological analyses

Soil microcosms were excavated intact from the field site on four dates: November 16, 2001 (T_0), April 14, 2002 (T_1), September 4, 2002 (T_2) and June 6, 2003 (T_3). Microcosms were sealed in plastic bags and kept at 4 °C until processing and soil determinations. The initial soil excavation (T_0) removed 4

control microcosms that received no added substrate. In April 2002, 24 microcosms were excavated: undisturbed control ($n = 4$), disturbed control ($n = 4$) and the four litter treatments ($n = 16$). On T_2 and T_3, 16 microcosms were excavated representing the four litter treatments. Fungal hyphae and fine roots that had penetrated into the microcosms through screens were bulked with the soil after severing at the microcosm exterior edge. Each microcosm was processed by initially separating soil from the O and A horizons. The top 10 cm of the A horizon was studied. Soil from each horizon was then sieved using a 2 mm sieve to separate particulate soil material (> 2 mm) and bulk soil (< 2 mm). The resulting 2 size fractions for each horizon were homogenized and separately analyzed. Total soil C and N were measured using a CHN gas analyzer on soil subsamples from each size fraction of the O and A horizons (Nelson and Sommers 1982; Table 1).

Soil microbial biomass C, N, and ^{15}N contents were determined on soil subsamples using the chloroform-fumigation extraction (CFE) method (5-d chloroform period) on field-moist soil (Brookes et al. 1985a, b; Vance et al. 1987). Fumigated and unfumigated soil subsamples were extracted with 0.5 M K_2SO_4 for soluble N (10:1 and 5:1 extractant:dry soil ratio for O and A horizon soil fractions, respectively). Total organic C in CFE extracts was quantified on a total organic carbon analyzer (O-I Analytical Co., College Station, TX). Total organic N in control and fumigated CFE extracts were quantified after conversion of organic N and NH_4^+ to NO_3^- using the alkaline persulfate digest method of Cabrera and Beare (1993); inorganic N in CFE were quantified colorimetrically on an automated N analyzer (Lachat Instruments, Milwaukee, WI) (Mulvaney 1996). Microbial biomass C and N were calculated after subtracting the value of the controls and adjusted using a k_{EC} of 0.38 and a k_{EN} of 0.45 (Jenkinson 1988). Soil particle size distribution in the A horizon was 675 g sand kg^{-1}, 235 g silt kg^{-1} and 90 g clay kg^{-1}. In the A horizon, cation exchange capacity was 9.0 cmol kg^{-1} measured by barium acetate saturation and calcium replacement (Janitzski 1986). Soil pH was 5.9 and electrical conductivity was 0.25 dS m^{-1} in the A horizon.

Soil CO_2 and $^{13}CO_2$ efflux

Every 60–90 d during the 2 y study, soil CO_2 flux rates were determined for all treatments and the controls, using a LI-6400 portable infrared gas analyzer (LI-COR, Lincoln Nebraska) and a soil respiration chamber (LI 6400-9) that we modified for headspace gas collection (Torn et al. 2003). The $\delta^{13}C$ of soil CO_2 efflux was estimated according to the Keeling plot method (Keeling 1958), based on five time points per respiration measurement. The $\delta^{13}C$ signature was calculated as the y-intercept of the linear regression of $\delta^{13}C$ vs. $[CO_2]^{-1}$ for the five data points per plot (Torn et al. 2003). The rate of soil $^{13}CO_2$ efflux is referred to as heterotrophic respiration of added ^{13}C.

Isotope analyses

Natural abundance values ($^{13}C/^{15}N$) of plant, soil, and microbial biomass were determined from plants collected onsite in 2001 and soil sampled on T_1. Natural abundance soil and plant samples were analyzed on a Europa Scientific Hydra 20/20 Isotope Ratio Mass Spectrometer (IRMS) (PDZ Europa, Cheshire, UK). Labeled and natural abundance inorganic N extracts (NO_3^-) from microbial biomass digest extracts were diffused onto ashed Whatman GF/A filter paper using the teflon tape method described by Stark and Hart (1996). For labeled samples, isotopic enrichment of the ^{15}N in diffused samples, and ^{13}C and ^{15}N in whole soil and plant were determined on a Europa Scientific INTEGRA IRMS (PDZ Europa, Cheshire, UK).

To measure ^{14}C content, organic soil C was reduced to graphite in three steps: complete combustion to CO_2 in the presence of CuO, cryogenic purification of CO_2, and sealed-tube zinc-reduction to graphite (Vogel 1992). Radiocarbon content was determined by Accelerator Mass Spectrometry at Lawrence Livermore National Laboratory, with precision better than $\pm 5.7‰$ of $\Delta^{14}C$ (Stuiver and Polach 1977). For each soil fraction, we estimated the mean C residence time with a one-pool stock-flow model (Trumbore 1993; Gaudinski et al. 2000; Torn et al. 2002) in which soil ^{14}C in year t is calculated as soil ^{14}C in the previous year plus the difference between the ^{14}C added in plant inputs and that lost via decomposition and radioactive decay. At steady state, the model can be simplified to Eq. (1). The spike of atmospheric ^{14}C from nuclear weapons testing is a good tracer of decadal turnover times, but when this approach does not provide a unique estimate of turnover time, NPP-stock relationships may be used to eliminate one option (Torn et al. 2005). In this study, for the A > 2 mm fraction, turnover times of 7 and 49 y were consistent with the ^{14}C data, but the 7 y turnover time was a better fit with site NPP and stock data

$$R_i = R_{i-1}(1 - \Delta t(\lambda - 1/\tau)) + \left(R_{\text{atm},i\text{-lag}}\right)\Delta t/\tau \quad (1)$$

where R_i = ^{14}C content of soil organic matter (SOM) in year i; Δt = annual time step; λ = ^{14}C radioactive decay constant (0.000121 y^{-1}); τ = turnover time of SOM (y). R_{atm} = ^{14}C of northern hemisphere atmospheric CO_2 in year i-lag. lag = years between the C being photosynthesized and entering the litter or soil (Torn et al. 2005); ponderosa pine needles typically live 3 y before entering O horizon. We assumed lag = 3 y for soil fraction O > 2 mm, and 5 y for all other soil fractions.

Statistical analyses

Main effects of litter type and soil depth were tested using a general linear model (GLM). All data are expressed as least squares means with standard

errors of indicated treatments. Fisher (F) statistics and p values are indicated in text and tables for all GLM procedures. A significance level of $p < 0.05$ was set *a priori* as the α-level; and $p > 0.10$ are noted as non-significant (NS). *Post-hoc* Tukey pairwise comparisons were performed for selected data. Studentized t-tests were performed among soil fractions on specific dates. Since proportional data are often not normally distributed and isotope recovery data often has nonhomogeneous variance, data were tested using Cochran's test for homogeneity of variance, and analyses were performed after log-transformation when needed. Total recovery of ^{13}C in soil was fit to a single exponential decay (SED) and an additive, double-exponential decay (DED) model for the first 1.5 y *in situ*. Parameters for the models include the initial pools size (S_1) and the overall decay rate (k_1) for the single exponential mode (Eq. (2)); and the pool sizes for the labile (S_1) and resistant components (S_2) and k_1 and k_2 (decay rates for the respective S_1 and S_2 pools; Eq. (3)). All statistical tests were performed using SYSTAT version 10.2 (SYSTAT Software Inc., Point Richmond, CA).

$$^{13}C(S_t) = S_1 e^{(-k_1 * t)} \tag{2}$$

$$^{13}C(S_t) = S_1 e^{(-k_1 * t)} + S_2 e^{(-k_2 * t)} \tag{3}$$

Results

C Dynamics

After 1.5 y *in situ*, the total retention of fine root ^{13}C (70.5 ± 2.2% of applied) exceeded that of needles (42.9 ± 1.3%) in microcosms ($F = 160$; $p < 0.001$), and was unaffected by the placement depth of substrates in the soil (Figure 2). This slower breakdown of fine root biomass was mainly in the particulate soil fraction (> 2 mm). Particulate fine root ^{13}C retention was almost double that of needles after 1.5 y (50.6 ± 2.2% compared 27.3 ± 2.8% of applied; $F = 47$; $p < 0.001$). For litter substrates applied to the A horizon, fine roots consistently had greater ^{13}C remaining in soil compared with needles during the 2 y study. However, 5 mos. after application, a litter type by soil depth interaction was present for total ^{13}C recovery in the soil ($F = 5.1$; $p = 0.049$). Specifically, fine roots decomposing in the O horizon lost more ^{13}C than those in the A horizon, but no depth effect was seen for needles (Figure 2). The recovery of ^{13}C in the bulk soil (< 2 mm soil fraction) was similar among treatments on all three excavation dates (Figure 2).

An additive DED function produced a better fit to the soil ^{13}C kinetics than a SED function (Table 3). For both substrates, the DED model described a labile pool that was much smaller than the more recalcitrant pool. The DED model described a smaller initial pulse of C loss from fine roots (S_1k_1) and a

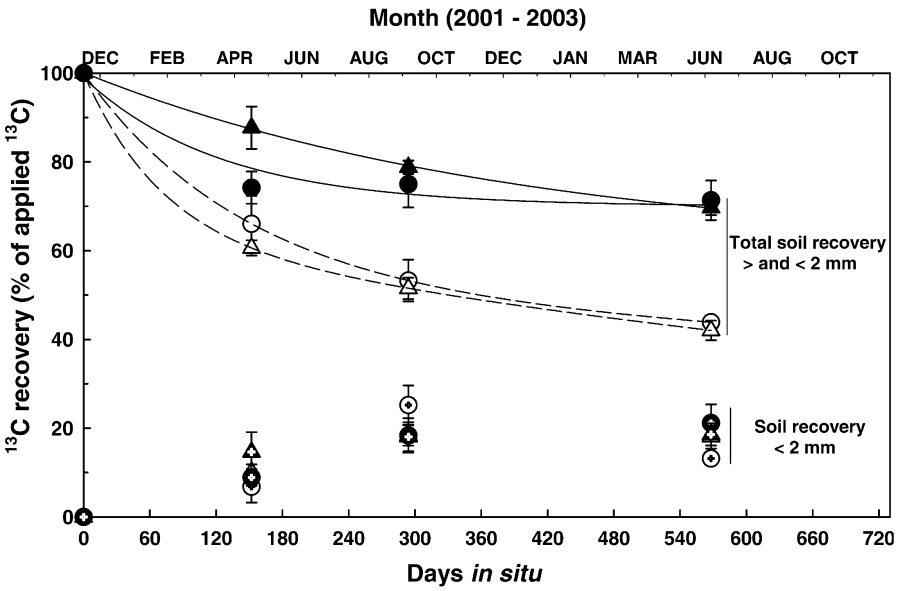

Figure 2. Total recovery of applied ^{13}C from needles (clear symbols) and fine roots (black symbols) applied to either 2–4 cm below the surface of the O horizon (circles) or 2–4 cm below the O/A horizon interface (triangles) during the first 2 y *in situ*. The percentage of ^{13}C recovered in the bulk soil (<2 mm size fraction) is shown with the cross-hatched symbols. Lines represent the results of an additive double exponential decay function for total ^{13}C recovery (Table 3). Errors shown are standard errors ($n = 4$).

slower decay rate of the recalcitrant ^{13}C pool for fine roots (0.03 y^{-1}) compared with needles (0.19 y^{-1}, averaged across depths).

Soil $^{13}CO_2$ efflux was much faster from needles compared with fine roots during the first 5 mos. *in situ* (Figure 3; Table 4). After 5 mos., however, there were no significant differences in ^{13}C respiration rates between needles and fine roots. The depth of substrate placement was a more consistent determinant of ^{13}C respiration rates; however, the direction of the effect of soil depth was seasonally dependent (Figure 3; Table 4). In the moist winter and spring, soil $^{13}CO_2$ efflux from both litters applied to the O horizon exceeded that from the A horizon. In contrast, during the drier summer and autumn period, soil $^{13}CO_2$ efflux from litter substrates applied to the A horizon exceeded that from the O horizon (Figure 3; Table 4). Total soil CO_2 efflux (i.e., autotrophic and heterotrophic unlabeled CO_2) was not affected by treatment or the addition of litter material and disturbance (Figure 3).

Cumulative ^{13}C loss as respired $^{13}CO_2$ was estimated over the first 1.5 y by integrating the soil $^{13}CO_2$ efflux data presented in Figure 3, assuming no efflux on the day of application. By this method, needles lost 42.7% of C when applied to the O horizon and 24.7% when applied to the A horizon. By

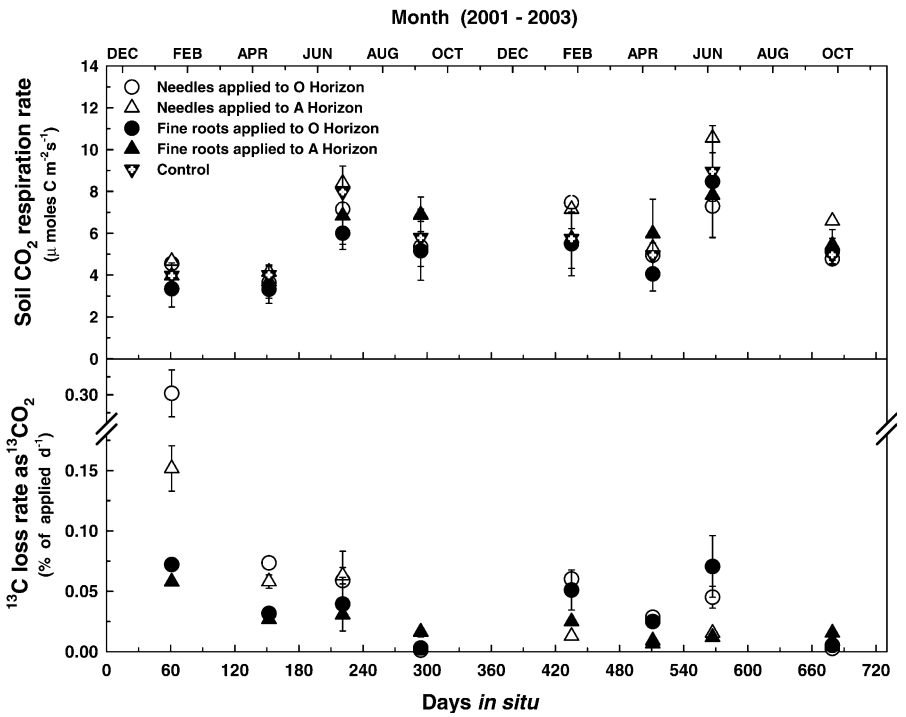

Figure 3. Soil CO_2 efflux during the 2 y field experiment (top). Control plots shown received no addition of needles or fine roots and were undisturbed. Needles or fine roots were applied to the O or A horizon. Loss rate of applied ^{13}C as soil $^{13}CO_2$ is expressed as percent loss per day during the 2 y after substrate addition (bottom). Errors shown are standard errors ($n = 4$).

contrast, roots lost much less: 20.8% when applied to the O horizon and only 14% when applied to the A horizon.

N Dynamics

Litter ^{15}N retention in microcosms was high (87.9 ± 2.1% of applied after 1.5 y) and unaffected by treatments on all three sampling dates (data not shown). The transformation of applied N into microbial biomass was determined for each soil size fraction except the A > 2 mm size fraction (due to its small mass). The microbial biomass C and N pools were 141 and 44% larger in the O horizon than the A horizon on a volumetric basis, respectively (Table 5). The proportion of soil-recovered ^{15}N immobilized into microbial biomass was greater in the O horizon than the A horizon on all 3 excavation dates for both litter types ($p < 0.05$; Figure 4). More ^{15}N from needles was recovered in the bulk soil (< 2 mm) microbial biomass for both soil depths after 5 mos. In the O horizon, ca. 12% more ^{15}N was immobilized into the microbial biomass at 10 mos. in both soil size fractions (Figure 4).

Table 3. Recovery of ^{13}C in soil (S_1 and S_2), decay rates (k_1 and k_2), and half-lives of ^{13}C over the 1.5 y period calculated using a single and double exponential equation (Eqs. (1) and (2)).

Litter substrate	Applied to horizon	Regression parameters						
		S_1 (% of added)	k_1 (y^{-1})	$t_{1/2}$ (S_1) (y)	S_2 (% of added)	k_2 (y^{-1})	$t_{1/2}$ (S_2) (y)	adj r^2
Double exponential decay								
Needles	O	48.4	2.5	0.28	51.6	0.12	5.8	0.88
Needles	A	37.2	5.2	0.13	62.6	0.26	2.7	0.97
Fine roots	O	26.1	6.8	0.10	73.8	0.03	26.4	0.62
Fine roots	A	35.0	1.0	0.69	65.1	0.03	24.6	0.82
Single exponential decay								
Needles	O	100	0.69	1.00				0.68
Needles	A	100	0.73	0.95				0.73
Fine roots	O	100	0.29	2.39				0.18
Fine roots	A	100	0.26	2.67				0.80

Functions fit using all treatments ($n = 4$).

Vertical movement of C and N

During the 2 y study, very little ^{13}C or ^{15}N was recovered outside of its placement soil horizon. No effect of litter type was observed for C or N recovered in the soil horizon below or above its original placement depth. Recovery of ^{13}C retained outside its placement depth was not different from zero at 5 mos. and was less than 1% of applied at 10 mos. and 1.5 y (Table 6). The recovery of ^{15}N moved into the O from the A horizon averaged 2.0% of applied (averaged across litter types). Movement of ^{15}N down into the A horizon increased over the 1.5 y period from $0.6 \pm 0.3\%$ at 5 mos. to $2.9 \pm 0.2\%$ at 1.5 y (Table 6). A substantial proportion of vertically moved soil ^{15}N was recovered as microbial ^{15}N (ranged from 4 to 28% of ^{15}N moved; data not shown).

Table 4. Summary of statistical parameters of main effects and interaction of the loss rate of applied ^{13}C as $^{13}CO_2$ during the first 2 y *in situ*.

Treatment effect	Statistic	Sample date (days after ^{13}C applied)							
		61	152	221	294	435	511	567	679
Litter type	F	82.3	60.5	2.77	0.78	0.05	1.28	0.52	1.52
	p	< 0.001	< 0.001	0.130	NS	NS	NS	NS	NS
Soil depth	F	17.0	4.62	0.02	35.5	13.8	42.9	19.8	6.38
	p	0.003	0.060	NS	< 0.001	0.005	< 0.001	0.003	0.032
L×SD	F	8.90	1.33	0.17	0.15	0.63	0.10	1.45	0.01
	p	0.015	NS	NS	NS	NS	NS	NS	NS

Means and standard deviations shown in Figure 3 (bottom).

Table 5. Microbial biomass C, N, and C:N ratios in the O horizon (> and <2 mm fractions) and A horizon (<2 mm fraction) as determined by fumigation–extraction.

Soil horizon	Size fraction	Microbial biomass		
		C (μg cm^{-3})	N (μg cm^{-3})	C:N
O	>2 mm	455 (24)	31.0 (1.4)	13.7 (0.6)
O	<2 mm	283 (13)	32.9 (1.7)	8.7 (0.3)
O	Total	738 (27)	63.9 (3.0)	11.8 (0.6)
A	<2 mm	306 (22)	45.0 (2.3)	6.9 (0.5)

Means from soil sampled in Apr. 2002; standard errors are shown in parentheses (n = 16).

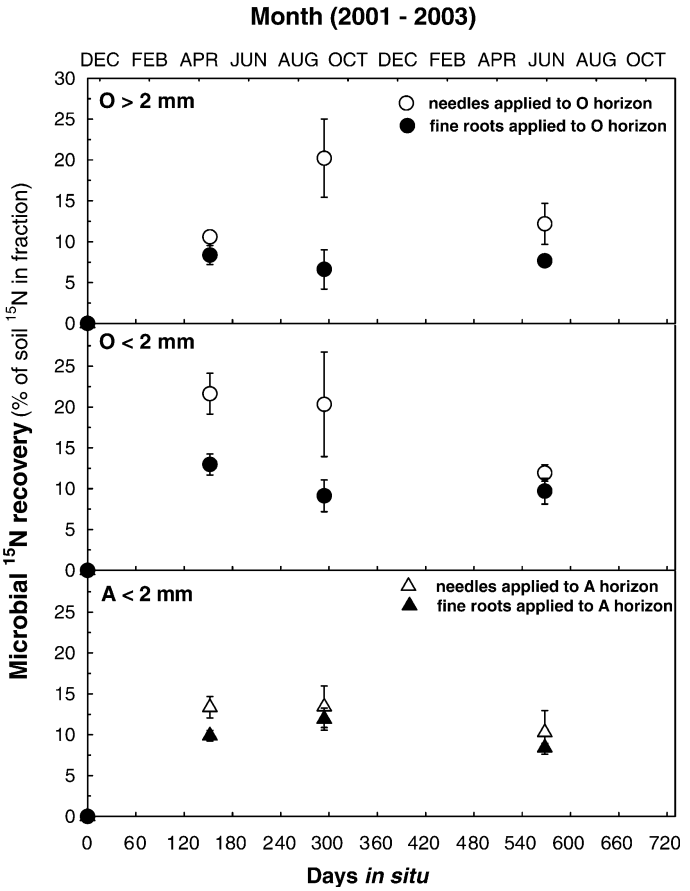

Figure 4. Microbial biomass ^{15}N from added needles (clear symbols) and fine roots (black symbols) expressed as the percentage of soil ^{15}N remaining in the particulate O horizon (>2 mm fraction; top), bulk O horizon soil (<2 mm fraction; center), and bulk A horizon soil (<2 mm fraction; bottom). Errors shown are standard errors (n = 4).

Table 6. Recovery of ^{13}C and ^{15}N from fine roots and needles moved either up or down from the soil horizon litter was placed (i.e., O to A or A to O), averaged across litter substrates.

	Days after application		
	152 (% of applied)	294 (% of applied)	568 (% of applied)
Moved from O to A horizon			
C	< 0.01 (0.04)	0.96 (0.37)	0.60 (0.44)
N	2.19 (0.70)	1.89 (0.76)	1.91 (0.45)
Moved from A to O horizon			
C	−0.10 (0.65)	0.76 (0.34)	0.96 (0.30)
N	0.57 (0.28)	1.41 (0.29)	2.91 (0.20)

Standard errors are shown in parentheses ($n = 8$).

Discussion

Litter type: fine roots vs. needles

In this 90-year-old temperate forest, belowground C allocation in *Pinus ponderosa* to fine roots greatly enhanced soil C retention after 1.5 y *in situ* compared with aboveground C allocation to needles. The turnover of needle C was 52% greater than fine root C added to soil. The lower extent of fine root decomposition was clearly evident in the reduction in particle size of needle C compared with fine roots. Moreover, more of the needle N remaining in the soil had been transformed into microbial biomass than for fine roots, regardless of soil depth. A greater degree of litter C and N transformation into microbial biomass may enhance stabilization of C and N in soil, because microbial residues are considered important components of stable soil organic matter associations (Kögel-Knabner 2002).

The relative difference between decomposition rates of above- and belowground litter can be illustrated by the ratio of their decay constants (i.e., needle or leaf k/fine root k). A recent analysis of temperate conifer and broadleaf forests ($n = 8$) showed first-order rate constant ratios from 1.4 to 1.9, suggesting slower C turnover rates for C allocated belowground to fine roots than for needles or leaves (Gholz et al. 2000). In our study, the aboveground to belowground k ratio was 0.99 for the smaller, labile pool (S_1) of the DED model, and 6.3 for the larger, resistant C (S_2) pool. The first-order ratio of aboveground to belowground rate constants was 2.5. The higher ratios we found show a wider gap between the turnover rates of aboveground and belowground litter in this ponderosa pine forest than has been observed in other temperate forest ecosystems, even for conifer forests with similar litter types (Gholz et al. 2000).

Like the DED expression of loss, litter decomposition and transformation is often described as a two-stage process: an initial stage involving relatively more rapid C loss rates of labile constituents and a slower, longer period, in which much of the remaining C is recalcitrant, complex plant C (e.g., polyphenols,

hemicellulose-lignin) or has been transformed into microbial biomass and humic substances (Paul and Clark 1996). In this study, the first stage of decomposition occurred during the first 5 mos. and was clearly driven by differences in labile C content between fine roots and needles. After 5 mos., litter type did not affect the rate of heterotrophic respiration (soil $^{13}CO_2$ efflux). Litter quality indices are major drivers in most decomposition models for both needles (Aerts 1997) and fine roots (Silver and Miya 2001). Indices that generally retard decomposition include low Ca, a high proportion of acid-resistant compounds, and high ratios of C:N or lignin:N. Consistent with these quality indices, pine fine roots evaluated in this study had a higher proportion of acid-hydrolyzable fraction (AHF)-C, acid-resistant fraction (ARF)-C, and higher C:N and ARF:N ratios than needles. Recent studies found that the mass loss of aboveground allocated C in needles/leaves was generally faster in the first 1–2 y than belowground allocated C (fine roots), in part, caused by differences in litter quality indices (Taylor et al. 1991; Gholz et al. 2000; Loya et al. 2004). A few studies have reported slower mass loss from leaves than fine roots (Hobbie 1996; Ostertag and Hobbie 1999); however, in these studies, the faster decay rates of fine roots correlated with the higher quality of fine roots than of leaves. Litter chemistry indices appear to be a more important predictor of C cycling rates than above- vs. belowground litter type *per se*.

The ^{13}C loss rates for both fine roots and needles were faster than, but similar in magnitude to, litter decay rates from conifer forests in Mediterranean areas, where moisture and temperature are negatively correlated and limit decomposition processes (Hart et al. 1992; Aerts 1997; Rovira and Vallejo 1997; Kurz et al. 2000). Most studies, however, measured litter decay by estimating mass loss (i.e., litter retained in mesh bags over time). Theoretically, mass loss should be faster than C loss rates from soil because mass loss methods do not consider litter C that has been transformed from its original structure, but is still retained in the soil. Alternatively, mass loss methods can underestimate decomposition rates because they limit contact between the soil and the substrate (Dornbush et al. 2002). In a 100-year-old forest stand near our study location, Hart et al. (1992) reported a first-order k rate constant of 0.08 y^{-1} for ponderosa pine needle mass loss, which is almost nine times slower than our first-order k for needle C (0.71 y^{-1}, averaged across depth). *Pinus pinaster* also produced a relatively slow first order decay k of 0.15 y^{-1} in Mediterranean conifer forest in France (Kurz et al. 2000). The mean decay constant assembled by Silver and Miya (2001) for conifer fine root mass loss was 0.17 ± 0.02 y^{-1} from 10 studies, which is slower than the average fine root ^{13}C first-order k of 0.28 y^{-1} found in this study. Recently, Girisha et al. (2003), using a DED model, reported slightly slower k_1 (0.58 y^{-1}) and faster k_2 (0.23 y^{-1}) for mass loss of *Pinus radiate* needles in New Zealand than the k_1 (3.8 y^{-1}) and k_2 (0.19 y^{-1}) reported here, but did not report the pool sizes (S_1 and S_2). Our faster decay rates may be because we placed needles and fine roots slightly below the litter surface (i.e., 2 cm below the top of the 9 cm deep O horizon), while many studies placed litter on the surface of the litter layer. The

slightly moister conditions 2–4 cm below the surface of the O horizon may have increased our turnover rates compared to the surface of the litter layer. Another reason for our faster rates may be because litterbags can retard the rate of C and N mineralization of litter due to limited litter to soil and decomposer contact.

Soil depth of litter placement

Soil depth effects were observed seasonally in microbial biomass and respiration rates, but did not result in differences in total ^{13}C recovery of both litters after 1.5 y due to offsetting seasonal trends. The climate had a greater effect on soil temperature and moisture in the O horizon than the underlying, insulated A horizon. The well-developed O horizon was warmer but much drier than the A horizon in the summer and autumn. During the dry periods of the year, heterotrophic respiration from the O horizon dropped to very low levels, well below rates in the A horizon. In Mediterranean climates, soil temperature and soil moisture content are asynchronous, causing dry soil conditions during the time of optimum temperature, thereby limiting C turnover. At this site, soil moisture content in the O horizon was related to rates of soil $^{13}CO_2$ efflux by a log function, which suggests a critical volumetric moisture content level, near 0.1 $m^3\ m^{-3}$, below which heterotrophic respiration rates declined significantly (Bird, unpublished data).

During the moist winter and spring periods, in contrast, heterotrophic respiration from the O horizon was greater than that from the A horizon for both litter types, even with colder soil temperatures in the O horizon. The larger microbial biomass in the O horizon ($\sim$2 times larger on a volumetric basis) compared with the A horizon may explain the greater soil heterotrophic respiration rates when moisture was not limiting, regardless of lower temperatures. Soil climate can affect decomposition rates, primarily by affecting the activity of the microbial community and its enzymes. Microbial biomass C and N in the O horizon decreased significantly during the summer period, which coincided with declining heterotrophic respiration rates in the O horizon. Unlike the O horizon, the size of the microbial biomass and heterotrophic respiration rates in the A horizon were similar during the year. The contrasting soil microbial community compositions in the two soil horizons, with the O horizon more fungal-dominated compared with the A (i.e., higher microbial C:N ratio in O), may have affected these patterns because of differences in substrate utilization efficiency.

Integrated soil $^{13}CO_2$ efflux over the course of the study provides an estimate of total ^{13}C loss via heterotrophic respiration. This approximation underestimated total ^{13}C loss compared with total soil recovery data by 24% for needles and by 27% for fine roots applied to the O horizon. Estimates of total ^{13}C loss using heterotrophic soil respiration were much poorer for both litter types in the A horizon, underestimating total ^{13}C loss by 54–58%. Certainly, a simple

integration of a limited number of heterotrophic soil respiration measurements should be treated with caution, especially since the first measurement was not until 2 mos. after litter application. The larger underestimate of total ^{13}C loss for substrates decomposing in the A horizon, however, suggests that there may have been certain times of the year when heterotrophic respiration rates were substantially higher in the A horizon than in the O horizon. One critical period of omission was likely during soil wet-up in the autumn (0–60 d). During the first few precipitation events, much of the water bypasses the O horizon due to its hydrophobic nature, especially its fungal mat at 2–6 cm. Since wet-up occurred just after substrate application, a portion of soluble litter–^{13}C in the A horizon may have been rapidly mineralized to $^{13}CO_2$. We hypothesize that during this initial wet-up period, soil $^{13}CO_2$ efflux from A horizon exceeded that from the drier O horizon. This trend would have been subsequently offset by the faster ^{13}C respiration rates observed from the O horizon than the A after the entire profile became moist.

The turnover of SOM generally slows with increasing soil depth, and has been attributed to limitations in oxygen and nutrients and the stabilizing influence of mineral assemblages (Trumbore 1993; Torn et al. 1997; Van Dam et al. 1997). Root decomposition rates have also been shown to decline significantly with increasing depth (Gill and Burke 2002). However, conditions in surface soil horizons may slow C turnover rates due to soil moisture constraints. For example, Rovira and Vallejo (1997) reported higher C and N recovery from pine needles at a 5 cm depth than at 20 cm. We observed a decline in ^{13}C respiration from the O horizon due to soil moisture limitation in the summer-fall; however, overall C loss was offset by faster respiration rates during winter–spring.

Only very small amounts of ^{13}C or ^{15}N (0–3% of applied) added to one soil horizon moved to the other (i.e., O to A or A to O). While our three measurement time points during 1.5 y are not a comprehensive estimate of leaching losses, they do (along with the integrated heterotrophic respiration and the high degree of total ^{15}N retention in the soil) suggest that most of the ^{13}C loss was via heterotrophic respiration. Transport of ^{15}N from litter in the A horizon into the O horizon was similar to the amount of ^{15}N moved from the O horizon to the A. This result suggests that the O horizon may provide a sink for potentially leachable N due to the activity of fungi translocating N and thereby buffering N losses from the A horizon. Litter-N loss from the soil most likely resulted primarily from leaching of dissolved inorganic and organic N, since reported denitrification rates are very low in this forest (0.1 kg N ha^{-1} y^{-1}) because of moisture and nitrate limitations (Strauss 1982; unpublished MS thesis).

Implications for long-term C sequestration

Our findings support an increase in the short-term soil C storage potential of this ecosystem if more plant C is allocated belowground to fine roots instead of

to needles. Caution should be taken in applying these results to C sequestration strategies for temperate forests that promote C allocation belowground. The large proportion of C retained in the particulate soil fractions (> 2 mm O and A) show that much of the added C is still in a form similar to its original structure. Hence, our findings must be regarded as interim because we have established C dynamics only over the initial decomposition and stabilization processes. In fact, there is isotopic evidence that slow initial C loss rates do not result in greater soil C sequestration in the long term (Voroney et al. 1989; Ladd et al. 1995). A 10 y comparison of C dynamics between a very labile C source (glucose) and low-quality C substrate (wheat straw) reported faster loss of glucose C in the first 2 y, but similar amounts of glucose-derived C and straw-derived C remained in the soil after 10 y (Voroney et al. 1989). A mechanism proposed for the slower than expected turnover of relatively labile C on the decadal scale is the greater proportion of microbial biomass formed from labile C, whose byproducts may be selectively stabilized in soil (Kögel-Knabner 2002).

The effect of soil depth may play a more important role at later stages in the litter decay and transformation processes. According to ^{14}C analysis of soil by depth (Table 1) native C in the O horizon turns over more quickly on average than in the A. As a result, it might be expected that litter C applied to the A horizon would have a longer residence time. Our data may be too preliminary to evaluate that because after 1.5 y, only 18% of ^{13}C had entered the bulk soil (< 2 mm soil fraction) for litter applied to the A horizon. The impact of differences between the O and A horizons, in microbial biomass residues, metal oxides concentrations, and mineral structure may not be observed fully until after more complete decomposition has occurred.

While the isotope approach used in this study provides some advantages over mass loss techniques, it also shares some important methodological artifacts. Potentially most importantly, the fine roots, while unwashed and well mixed with the soil and other litters, did not decay in intimate contact with their natural rhizosphere soil (Loya et al. 2004) and were not apparently infected by mycorrhizae, which may have slowed decomposition rates (Langley and Hungate 2003). Advantages of the tracer isotope method, despite the higher costs associated with substrate production and analyses, include a direct measure of mineralization, decomposition, and humification rates *in situ* without modifying pedoclimate; maintaining litter contact with soil, fauna, and other litter; and the ability to examine the degree of transformation into microbial and SOM pools.

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