LETTERS
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Formation of soil organic matter via biochemical and physical pathways of litter mass loss

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Soil organic matter is the largest terrestrial carbon pool[1]. The pool size depends on the balance between formation of soil organic matter from decomposition of plant litter and its mineralization to inorganic carbon. Knowledge of soil organic matter formation remains limited[2] and current C numerical models assume that stable soil organic matter is formed primarily from recalcitrant plant litter[3]. However, labile components of plant litter could also form mineral-stabilized soil organic matter[4]. Here we followed the decomposition of isotopically labelled above-ground litter and its incorporation into soil organic matter over three years in a grassland in Kansas, USA, and used laboratory incubations to determine the decay rates and pool structure of litter-derived organic matter. Early in decomposition, soil organic matter formed when non-structural compounds were lost from litter. Soil organic matter also formed at the end of decomposition, when both non-structural and structural compounds were lost at similar rates. We conclude that two pathways yield soil organic matter efficiently. A dissolved organic matter–microbial path occurs early in decomposition when litter loses mostly non-structural compounds, which are incorporated into microbial biomass at high rates, resulting in efficient soil organic matter formation. An equally efficient physical-transfer path occurs when litter fragments move into soil.

Soil organic matter (SOM) is formed through the partial decomposition and transformation of plant inputs (for example, above- and below-ground litter and exudates) by soil organisms. Although much is known about the factors controlling litter decomposition rates[5], we argue that these are of little importance to long-term net ecosystem C balance. Rather, what really matters is the proportion of plant inputs that are incorporated and stabilized into SOM versus the amount that is mineralized.

Litter chemistry is a primary controller of litter decomposition, with litter characterized by low N and high lignin content generally described as recalcitrant, that is, showing low decomposition rates and high relative amounts of remaining residue[6,7]. Yet, the amount of stable SOM is now thought to be largely independent of litter recalcitrance[8], and most inputs into the SOM pool have already been processed by microbes[9,10]. Clearly we miss the nexus between plant input decay and SOM formation. Traditionally, for litter decomposition, the guiding paradigm has been that litter mass loss—measured using mesh bags—corresponds to C mineralization, whereas the fraction of litter that does not decay at a measureable rate (that is, mass remaining) contributes to SOM formation[11]. As a consequence, any factor that slows mass loss and/or increases the amount of mass remaining should favour SOM formation. Following this paradigm, most litter decomposition studies have focused on decay rates and mass loss dynamics[5], neglecting to quantify litter-derived C and N incorporation in the mineral soil while mass is being lost. Despite pioneering C isotope tracing studies presenting evidence of accumulation of labile compounds and microbial products in stable SOM forms[12,13], only recently has this idea found traction. Exciting studies have shown that in the early stages of decomposition, a surprisingly large amount of litter-C is sequestered in mineral soils[14,15], and dissolved organic matter (DOM) produced during litter decomposition can promptly associate with silt and clay fractions in the top soil mineral layer[16]. Products of microbial transformation of plant litter are likely to contribute more to stabilized SOM than do original plant litter compounds[17]. Recent work showing that lignin does not preferentially accumulate in decomposing litter[18] contradicts the long-standing paradigm that plant lignin is recalcitrant and suggests that it might actually decompose during all stages of decomposition[19,20]. Synthesizing this understanding, the microbial efficiency mineral stabilization framework[4], proposed that labile litter compounds would result in stable SOM formation in soils with high capacity for C stabilization (for example, fine texture, high sorption capacity, low C saturation) because microbes use them efficiently. Some initial support for this hypothesis exists in the literature[21,22], but it remains largely unexplored.

We followed the dynamics of carbon (C) and nitrogen (N) from a dual (3.38 atom% ^{13}C; 3.99 atom% ^{15}N)-labelled *Andropogon gerardii* above-ground litter in the tallgrass prairie through complete mass loss, and traced litter-derived C and N incorporation in SOM to a depth of 20 cm (Fig. 1 and Supplementary Fig. 1). Carbon was lost from the litter following a first-order exponential decay with rates of 0.809 yr^{-1} ($r^2 = 0.956$). Our litter had an initial N concentration of 1.46% and a C/N ratio of 30, and released endogenous N from the onset of decomposition (Supplementary Fig. 1), as expected[23]. Less expected was the complete mass loss in three years. Litter decomposition has been conventionally measured by the litter bag method, which by inhibiting fragmentation results in an 'artificial' asymptotic value of mass remaining on the soil surface[11]. When litter is not protected in mesh bags, it is fully exposed to biophysical perturbations that accelerate its rate of mass loss to full disappearance from the litter layer within a few years. Our study was conducted in a mesic environment where a previous litter bag study had observed 18–50% litter mass remaining after three years depending on residue chemistry[6]. Different litter chemistry and climatic

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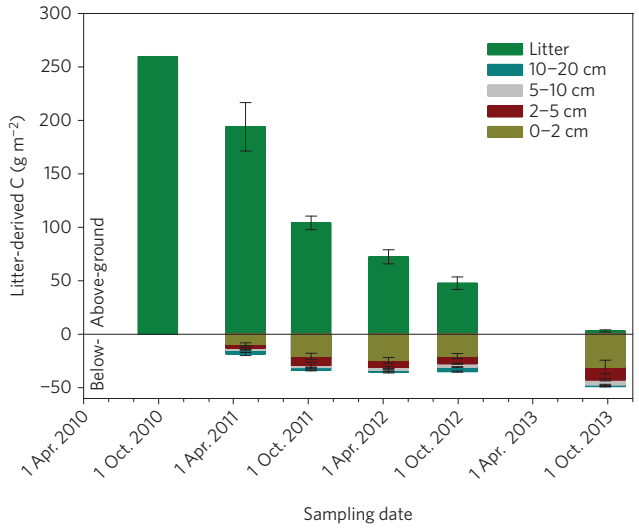

Figure 1 | Dynamics of *A. gerardii* litter C loss from the soil surface and recovery of litter-derived C in the mineral soil. In September 2010, the equivalent of 259 gC m^{-2} litter was placed on the soil surface, where it decomposed for three years with sampling at 7, 12, 18, 24 and 36 months. At each sampling the amount of litter C remaining on the soil surface and recovered in the 0–2, 2–5, 5–10 and 10–20 cm mineral soil layer was quantified by applying the isotopic mass balance to the litter and soil C pools. Data are means ($n=4$) with standard errors.

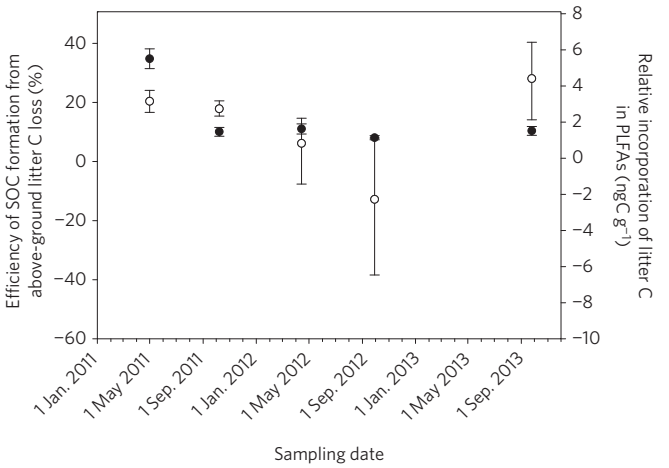

Figure 2 | Efficiency of SOC formation from litter C loss, and relative microbial incorporation of litter-derived C during *A. gerardii* litter decomposition. SOC formation efficiencies (open circle, left *y* axis) are calculated as the percentage of SOC gain/litter C loss, and are high at the beginning and end of the decomposition process. Data are means with 95% confidence intervals, except for the first sampling when data are mean ± 2 s.d. Relative microbial incorporation of litter-derived C (filled circle, right *y* axis) is calculated as litter-derived PLFAs-C/litter-derived SOC, and is high only at the beginning of the decomposition process. Data are means with standard errors ($n=4$).

conditions will require different amounts of time for complete litter mass loss. Our study demonstrates that existing values for surface litter decay rates may be underestimated, and that more accurate values from studies where leaching and fragmentation are not inhibited are necessary.

Litter-derived C was retrieved in the mineral soil to 20 cm depth (Fig. 1; we explored deeper depths but did not find the isotope label). As early as seven months following litter incubation, and throughout the first year, we found a high efficiency of SOM formation (that is, litter-derived C in SOM divided by C lost from the litter; Fig. 2). Intermediate stages of relatively little mass loss did not produce a net accrual of new SOM, whereas during the last year litter C moved again to the soil where it was recovered with high efficiency (Fig. 2). By the end of the experiment, on average 19% of the initial C was retrieved in SOM. This value is well within the range (0–33%) of recently reviewed plant litter to SOM conversion rates[24].

To assess what generated the observed dynamic of SOM formation from above-ground litter decomposition we investigated the changes in litter residue chemistry and determined the amount of specific litter constituents lost through time. We also estimated the microbial incorporation of litter-derived C in soil and, in a laboratory experiment[25], the potential partitioning of C loss from *A. gerardii* litter between dissolved organic carbon and CO_2. During the early stages of decomposition, non-structural compounds were lost preferentially from the litter (Supplementary Fig. 2). The loss of these compounds is linearly related to the production of dissolved organic carbon, which overall accounted for over 1/3 of the C lost from *A. gerardii* litter during decomposition[25]. Nitrogen was also lost at a high rate at this early stage (Supplementary Fig. 1) while new soil organic carbon (SOC) was formed with high efficiency (Fig. 2). In contrast, the acid unhydrolysable fraction, generally defined as 'lignin', increased in absolute amounts, possibly owing to the incorporation of microbial residues in this fraction. We found the highest relative litter-derived C incorporation in microbial phospholipid fatty acids (PLFAs) in the 0–5 cm soil layer after the first sampling, whereas progressively lower incorporation values were measured in subsequent samples (Fig. 2). These results suggest that DOM leaching from litter followed by high microbial incorporation of this input is the dominant pathway of SOM formation from litter decomposition at the initial phase (Fig. 3). A highly efficient DOM–microbial pathway of SOM formation during the early stage of litter decomposition is consistent with recent theoretical work showing higher use efficiency of substrates rich in N and with a low lignocellulose index[26,27].

A different pathway seems to explain the high efficiency of SOM formation at the final stage of decomposition. At this stage there was no preferential loss of any chemical compounds (Supplementary Fig. 2), DOM leaching is less important[25] and soil microbes did not additionally incorporate litter C (Supplementary Fig. 3); yet, we observed the highest SOM formation efficiency in this period, following a period of no net new SOM accrual (Fig. 2). We suggest that at this late stage SOC incorporation occurred by physical transfer of the brittle litter residues, with little microbial processing in the soil (Fig. 3). The litter residue physically transferred to the mineral soil would explain the common asymptotic mass remaining found in litter bags studies, where loss of litter fragments is inhibited. In a previous study[6] conducted at our study site using litter bags, around 7% of the initial amount of litter was eventually found as the asymptotic value of mass remaining for a litter with a chemistry (11% lignin and a C/N ratio of 25) comparable to the one used in this study. This 7% mass remaining is similar to what we determined as the amount of litter-derived C mixed into the soil by physical transfer.

High efficiency of SOM formation does not necessarily correspond to high persistence of this new C in soil. However, when we examined the potential decay of the litter-derived C in the 2–5 cm soils collected after 12 and 24 months of litter decomposition by laboratory incubation, we found that 94% to 97%, respectively for the 12- and 24-month sample, of the litter-derived C in soil was relatively stable (Supplementary Fig. 4). Two mechanisms are likely to determine this potential stability. SOM formed through the DOM–microbial pathway can stabilize by organo-mineral interactions[28], whereas physically transferred litter residues are recovered as a light fraction in soil, and can be stabilized by inherent chemical recalcitrance, or eventually promote aggregation and be

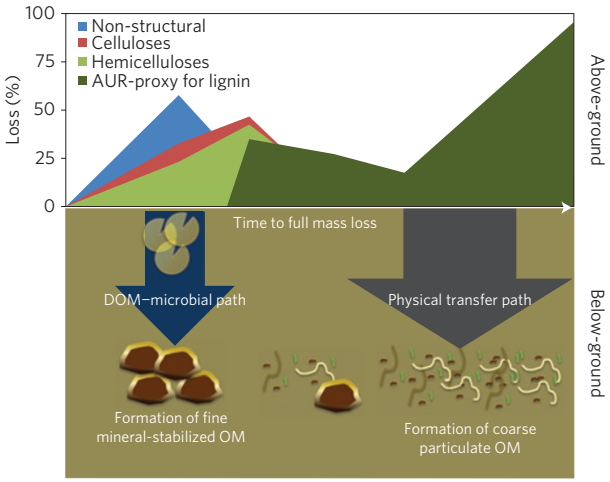

Figure 3 | Schematic representation of biochemical and physical pathways leading to SOM formation from progressive loss of litter chemical components. Each component loss is in percentage of its initial weight. Data are averages ($n=4$). Initially, the acid unhydrolysable fraction (AUR) gained mass; thus, its loss is higher than 100% initial weight. The DOM–microbial path is important during early stages of litter decomposition, when litter loses mostly non-structural soluble compounds that are efficiently incorporated by microbes, resulting in mineral-associated fine organic matter (OM). Later, brittle litter residues move in the mineral soil by physical transfer where they form coarse particulate organic matter.

protected by spatial inaccessibility[29]. The stabilization potential and climatic conditions of the soil will ultimately determine how much of the litter-derived SOM will persist in the long term[4]. We quantified the litter-derived C incorporation in the light fraction ($<1.85\,g\,cm^{-3}$) and in the sand-size, silt and clay organo-mineral fractions of the top soil (0–5 cm) and found that although at the first harvest 68% of the litter-derived C in soil was recovered in the mineral-associated silt and clay fraction, by the final harvest most of the litter-derived C was in the light (44.3%) and sand-sized (21.5%) fractions (Supplementary Table 1). Fine textured soils, generally characterized by high sorption capacity and high aggregation—such as the one used in this study (see Supplementary Information)—are expected to initially stabilize litter-derived organic matter incorporation from both pathways. Yet, the longest time of persistence in soil (>100 years) is found for mineral-associated fractions[29]. Our findings of early-stage litter C loss contributing mostly to the mineral-associated SOM fraction (Supplementary Table 1) and that 94% of this litter-derived SOC is stable (Supplementary Fig. 3) support the hypothesis that labile litter components form the most stable SOM (ref. 4).

Our study demonstrated that in the tallgrass prairie a significant amount (19%) of above-ground litter C loss is not mineralized to CO_2 but transferred to the mineral soil where it contributes to the formation of SOM. We identified two dominant pathways of above-ground litter decomposition contributing to SOM formation: a DOM–microbial pathway, significant at the early stage of decomposition where labile litter components are decomposed, and a physical transfer of litter particulate matter at the final stage, whereas intermediate stages, characterized by the decomposition of structural compounds (for example, lignin and lignin encrusted cellulose) do not lead to new net SOM formation (Figs 2 and 3). These two pathways result in different mechanisms of SOM stabilization (Fig. 3): mineral association via the DOM–microbial path versus inherent chemical recalcitrance via the physical-transfer path, and both possibly physical occlusion. Traditional above-ground litter decomposition studies consider only the remaining particulate litter residue as contributing to stable SOM, underestimating C fluxes to the mineral soil during the initial stages of litter decomposition. We highlighted the importance of the DOM–microbial pathway for the formation of mineral-associated SOM and stress the need for both of these decomposition pathways to be determined in the field and explicitly represented in future biogeochemical models.

Methods

Methods and any associated references are available in the online version of the paper.

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Author contributions

M.F.C. developed the concepts, designed and led the research, contributed to data analyses and wrote the paper. J.L.S. and A.J.H. conducted all the research work and analyses. E.E.C. and M.L.H. contributed to data and statistical analyses. D.H.W. and W.J.P. were co-PI on the project and contributed to the development of research concepts. All authors contributed to writing of the paper.

Additional information

Supplementary information is available in the online version of the paper. Reprints and permissions information is available online at www.nature.com/reprints. Correspondence and requests for materials should be addressed to M.F.C.

Competing financial interests

The authors declare no competing financial interests.

Methods

Study site. The study was conducted at the Konza long-term ecological research station. The station occupies a topographically diverse area of 36 km^2 in the Flint Hills of northeastern Kansas, USA. It is a tallgrass prairie. Big bluestem (*Andropogon gerardii*) is the dominant grass at the site, with an average 79.4% ground cover[30,31]. Indian grass (*Sorghastrum nutans*), switchgrass (*Panicum virgatum*) and little bluestem (*A. scoparius*) comprise the remaining dominant vegetation at Konza, although there are numerous sub-dominant grasses, forbs and woody species as well. The mean annual precipitation at Konza is 835 mm and the mean annual temperature is 12.9 °C. Soils are predominantly fine textured silty clay loam, and are derived from limestone and shale parent material[30].

For the decomposition study a 20-year reoccurrence burning area, referred to as R20B, was used. This area burned annually from 1972 to 2000, when burning stopped with the exception of a wild fire in 2008. In a pre-sampling campaign soil bulk density (BD) in the experimental area was determined on 4 replicates by the soil core (5 cm) method, for the 0–2 cm (BD = 0.702 ± 0.05), the 2–5 cm (BD = 0.922 ± 0.04), the 5–10 cm (BD = 1.1 ± 0.24) and 10–20 cm (BD = 1.28 ± 0.11) depth layer. Soils were also tested for the presence of inorganic C by a pressure transducer following acid addition, but none was found.

Dual-labelled litter decomposition experiment. To trace litter-derived C and N in soils, a 3.3804 atom% ^{13}C- and 3.9917 atom% ^{15}N-labelled *A. gerardii* above-ground litter was used. The above-ground litter was harvested at early senescence from *A. gerardii* Kaw plants grown for 22 weeks from rhizomes under controlled conditions inside a continuous-labelling chamber, as described in ref. 32. Litter was air-dried, cut to 20 cm and well mixed in a homogeneous pool. Litter water content was measured on three oven-dried (65 °C) litter subsamples, for dry-weight correction. The oven-dried subsamples were pulverized and used for: the determination of C (44.3%) and N (1.47%) concentrations and their isotopic composition ($\delta^{13}C = 2{,}113‰$; $\delta^{15}N = 10{,}309‰$) by elemental analysis–isotope ratio mass spectrometry (EA–IRMS, Carlo Erba NA 1500 coupled to a VG Isochrom continuous-flow IRMS, Isoprime, at the EcoCore analytical facility, Colorado State University); and for the chemical fractionation of the litter mass into a non-structural (42.04%), a hemicellulose (25.11%), an α-cellulose (28.65%), an acid unhydrolysable (AUR 3.9%, generally referred to as lignin) and an ash (0.26%) fraction using conventional detergent fibre methods[33].

On 29 September 2010, the air-dry labelled litter was incubated on the surface of the tall grass prairie, inside PVC collars (∅20 cm) with 18.4 g dry-weight equivalent litter per collar, at direct contact with the ground; a PVC netting with a 6.35 mm mesh size covered the collars to prevent fresh litter inputs. Plants were previously removed from inside the collars by clipping and herbicide. The experimental design consisted of a litter treatment: with and without the dual-labelled litter (control bare soil), and time: five harvests that occurred on 1 May 2011 (7 months), 8 October 2011 (12 months), 13 April 2012 (18 months), 29 September 2012 (24 months) and 25 September 2013 (36 months), in a fully randomized block design with 4 replicate blocks.

At each harvest, a bare-soil control and a litter collar from each of the four replicate blocks were sampled. Litter was carefully handpicked and the soil was excavated by hand shovel into a 0–2, 2–5, 5–10 and 10–20 cm layer and all samples placed in pre-labelled plastic bags, and kept cool (4 °C) until used for further analyses, which always occurred within two weeks from sampling.

In the laboratory, litter samples were processed and analysed as described above for the initial litter samples. All soil samples were sieved to 2 mm, and air-dried. A representative subsample from each soil sample was dried in an oven at 105 °C, pulverized and used for elemental and isotopic analyses as described above for the litter. The 0–2 and 2–5 cm soils were processed for the determination of soil organic matter (SOM) physical fraction and microbial phospholipid fatty acid (PLFA). SOM was fractionated by size and density to separate its primary components[34], using the same procedure described in ref. 15. In brief, after dispersion SOM was separated into a light fraction (LF < 1.85 g cm^{-3}) and a heavy fraction. The latter was separated through wet sieving by size, into a sand-sized (>53 μm) and a silt-and-clay fraction (<53 μm), which was further separated into a silt-associated and a clay-associated organic matter fraction by centrifugation. This approach allows the separation of SOM into biologically meaningful fractions, at different degrees of decomposition and stabilization[9]. PLFAs were extracted from 2 mm sieved soil subsamples, cleaned of any residual fine root, frozen at −20 °C and lyophilized 48 h before extraction using conventional methods, as described in ref. 35. Phospholipids were used to obtain free fatty acids, and then free fatty acids were methylated using 0.2 M methanol KOH to form fatty acid methyl esters (FAMEs). FAMEs were analysed using a capillary gas chromatograph–mass spectrometer (Shimadzu QP-2010SE) with a SHRIX-5 ms column (30 m length × 0.25 mm ID, 0.25 μm film thickness). A capillary gas chromatography–combustion–isotope ratio mass spectrometer (GC-c-IRMS; Trace GC Ultra, GC Isolink and DeltaV IRMS, Thermo Scientific) was used to determine the C% and δ^{13}C of the FAMEs. To obtain δ^{13}C values of the PLFAs, measured δ^{13}C FAMEs values were corrected individually for the addition of the methyl group by simple mass balance[36].

Particular care was used from sampling to analyses to avoid isotopic contamination among samples. Isotope analyses suggested no occurrence of even slight contamination.

Laboratory incubation. Soil samples collected from the 2–5 cm layer at the 12- and 24-month harvests from the litter and control treatment collars of the dual-labelled litter decomposition experiment described above were used for a laboratory incubation to determine the pool structure and estimate decay rates of the litter-derived C in the soil. Air-dried soil samples (40 g) were moistened to 60% water-holding capacity and incubated inside 1.89 l air-tight glass jars with lids containing septa for gas sampling. Soils were incubated in a constant-temperature room at 25 °C in the dark for 150 days. Soil respiration was measured on day 1, 4, 11, 18, 32, 46, 60, 81, 112 and 150 of the incubation by determining the CO_2 concentration accumulated in the jar headspace during the time elapsed between the last sampling[37]. After mixing the jar's headspace, a 2-ml subsample was manually injected into an infrared gas analyser (IRGA, model LI6252, LICOR). Calibration with an external standard curve determined CO_2 concentration. On the same dates, 0.75 ml of headspace air was also injected into a VG Optima IRMS (Isoprime) with a microgas injector and equilibration block to determine the δ^{13}C value of the CO_2 (observed precision: 0.3‰).

Data analyses. To determine litter-derived C and N amounts in the bulk soils and SOM fractions, the average field pool size for each of those pools was determined (Supplementary Table 3). Data (%C, %N and % fraction) from the control collars averaged across all replicates and harvests, and bulk density data were used for this purpose.

The litter C and N contribution to the bulk soil, SOM fractions, PLFA-C and CO_2 was assessed using the isotopic mixing model:

$$f_{\text{litter}} = \frac{\delta_S - \delta_B}{\delta_{\text{litter}} - \delta_B}$$

where f_{litter} is the fraction of litter-derived C (or N) contributing to bulk soil, SOM, PLFA and CO_2 samples. The δ_S and δ_B are the δ^{13}C (or δ^{15}N) of the specific bulk soil, SOM, PLFA or CO_2 sample from the litter (δ_S) and the control (δ_B) treatment, respectively. For bulk soil and SOM, the δ_B average values across all bare collars are used (Supplementary Table 3); and δ_{litter} is the δ^{13}C (or δ^{15}N) of the initial litter.

Efficiencies of SOC formation were obtained as the ratio of isotopically labelled SOC change from 0 to 20 cm between harvests versus isotopically labelled litter C loss. The efficiency of SOC formation between the start of the experiment (only one value) and the initial harvest had a limited sample size; therefore, 95% confidence intervals (CIs) were approximated as the mean ±2 s.d. For SOC formation efficiencies between each subsequent harvest, 95% CIs were calculated using the bootstrap resample with replacement technique[38], applied to SOC formation efficiencies calculated from all possible combinations of replicates between harvests. This is based on the independence of replicates at each harvest date, calculating all possible combinations of replicates between sample periods as a measure of variation in SOC formation efficiencies. The highest and lowest efficiencies were trimmed for each harvest date to prevent skew from extreme values, yielding a final $n = 14$. The 'boot' package[39] in R (ref. 40) was then used with 1,000 replications to generate 95% CIs for each harvest date. Results were assessed for normality using quantile–quantile plots.

Relative microbial incorporation of the litter-derived C present in the 0–5 cm soil was calculated by summing the PLFA-litter-derived C pool (ng) for the 0–2 and 2–5 cm samples, by replicate and harvest, and dividing that by the sum of the litter-derived C pool (g) for the 0–2 and 2–5 cm samples of the correspondent replicate and harvest.

The litter chemical fraction lost between any two sampling times was calculated by subtracting from the average ($n = 4$) amount (g) of any chemical fraction at a time harvest the correspondent chemical fraction amount at the following harvest, by replicate ($n = 4$). Chemical fraction amounts were obtained by multiplying the chemical fraction concentration for the amount of mass recovered at each harvest, by replicate ($n = 4$). Because at the final harvest only less than 1 g litter residue dry weight remained, which was too little for chemical fractionation, the amount of each chemical fraction measured at harvest 4 (that is, 24 months) was considered lost by harvest 5 (that is, 36 months).

To determine the pool structure and persistence of the litter-derived C in soil, litter-derived CO_2 data were fitted by the following two-pool first-order equation: $\Delta C_{\text{cum}}/\Delta t = C_a k_a (e^{-k_a t}) + C_s k_s (e^{-k_s t})$, where $C_{\text{cum}}(t)$ is the cumulative soil respiration at time t (μgC g^{-1} soil), C_a is the size of the active fraction (μgC g^{-1} soil), and C_s is the size of the slow fraction (μgC g^{-1} soil). The parameters k_a and k_s are the decomposition rate constants for the active and slow pools, respectively[41]. Best fit parameters for the two-pool model were estimated using nonlinear regression of the CO_2 evolved with time in SAS v9.3 PROC NLIN with the Gauss method (SAS Institute) with the only restriction imposed on the parameters being that the values had to be greater than zero. A Q_{10} of 2 was used to correct the laboratory decay rate at 25 °C to the field mean annual temperature of 12.9 °C.

Model estimates were: $C_a = 6\%$ and $C_s = 94\%$, $k_a = 0.192\ d^{-1}$ and $K_s = 0.0005\ d^{-1}$ for the 12 month soil ($r^2 = 0.958$), and $C_a = 3\%$ and $C_s = 97\%$, $k_a = 0.204\ d^{-1}$ and $K_s = 0.0003\ d^{-1}$ for the 24 month soil ($r^2 = 0.972$).

Data availability. Data collected in this study are preserved in the Colorado State University digital repository and are accessible at: http://hdl.handle.net/10217/90205.

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