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Long term decomposition: the influence of litter type and soil horizon on retention of plant carbon and nitrogen in soils

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Abstract How plant inputs from above- versus below-ground affect long term carbon (C) and nitrogen (N) retention and stabilization in soils is not well known. We present results of a decade-long field study that traced the decomposition of ^{13}C- and ^{15}N-labeled *Pinus ponderosa* needle and fine root litter placed in O or A soil horizons of a sandy Alfisol under a coniferous forest. We measured the retention of litter-derived C and N in particulate (>2 mm) and bulk soil (<2 mm) fractions, as well as in density-separated free light and three mineral-associated fractions. After 10 years, the influence of slower initial mineralization of root litter compared to needle litter was still evident: almost twice as much root litter (44% of C) was retained than needle litter (22–28% of C). After 10 years, the O horizon retained more litter in coarse particulate matter implying the crucial comminution step was slower than in the A horizon, while the A horizon retained more litter in the finer bulk soil, where it was recovered in organo-mineral associations. Retention in these A horizon mineral-associated fractions was similar for roots and needles. Nearly 5% of the applied litter C (and almost 15% of the applied N) was in organo-mineral associations, which had centennial residence times and potential for long-term stabilization. Vertical movement of litter-derived C was minimal after a decade, but N was significantly more mobile. Overall, the legacy of initial litter quality influences total SOM retention; however, the potential for and mechanisms of long-term SOM stabilization are influenced not by litter type but by soil horizon.

Keywords Litter · Decomposition · 13C · 15N · Needle · Fine root · Soil organic matter · Stabilization · Density fractionation · Organo-mineral associations

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Introduction

The fate of plant inputs as they decompose determines soil fertility and soil organic matter (SOM) sequestration. Decomposition of plant litters as mass loss has been well-studied for a wide variety of plant species and in a range of ecosystems (Aerts 1997, 2006; Silver

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and Miya 2001); yet how plant litter inputs are transformed and retained as SOM, and in what form and quantity, is not well understood. Isotopically-labeled material has been used to study how plant litter transforms into SOM over 1–5 years of decomposition (e.g. Hatton et al. 2012; Cotrufo et al. 2015; Haddix et al. 2016). However, few studies (Voroney et al. 1989; Beyaert and Voroney 2011) have followed this transformation across a decade and into the later stages of decomposition to assess whether conclusions based on short-term studies can be extrapolated to longer timescales.

Bridging the gap between litter decomposition and SOM formation and long-term retention is critical. Although these processes form a continuum, the dominant paradigms in these two fields of study are seemingly diverging in part because studies of litter decay and SOM often occur separately (Cotrufo et al. 2013). Historically, differences in decomposability among litter types and SOM pools were primarily attributed to differences in their chemical composition (Lehmann and Kleber 2015). Many litter decomposition studies support this view, finding that litter type (Hobbie 1996; Dorrepaal et al. 2005) or chemistry (Melillo et al. 1982; Silver and Miya 2001; Wieder et al. 2009) affect decomposition rates. For example, roots generally decompose slower than foliar tissues (Bloomfield et al. 1993; Rasse et al. 2005; Fujii and Takeda 2010). However, it is now understood that SOM stabilization is not dominated solely by chemical recalcitrance but rather by the interactions of SOM, soil physical properties, microbial communities, mineralogy, and climate (Schmidt et al. 2011; Lehmann and Kleber 2015). In this view, the accessibility of SOM to microbes affects decomposition rates more than its chemical composition (Dungait et al. 2012). Furthermore, physical, chemical, and microbial soil properties and their interactions with SOM change with depth (Rumpel and Kögel-Knabner 2011). How the original identity of litter-derived SOM affects decomposition once it is part of soil matrices, both organic- and mineral-dominated, is an open question.

The diverging views of litter decomposition and SOM stabilization are not necessarily mutually exclusive and may be resolved by tracing litter decomposition over long timescales. The dominant controls over initial litter decomposition likely differ from the dominant controls over stabilization once litter-derived OM is within the soil matrix. Melillo et al. (1989) posited that initial litter decomposition was controlled by both litter quality and the environment but that later stages of decay after microbial processing were effectively controlled by the environment only. Confirmation that most of the organic compounds stabilized in the soil matrix are the product of microbial processing (Miltner et al. 2012) gave rise to the MEMS (Microbial Efficiency-Matrix Stabilization) framework, which predicts that more rapidly decomposed plant inputs are the dominant source of stabilized SOM (Cotrufo et al. 2013). In this view, microbial processing transforms litter-derived SOM into smaller molecules that can then be stabilized in the soil matrix via physical occlusion, organic-organic, and organo-mineral interactions. Thus SOM stabilization efficiency may be affected by the large differences in soil biota, microclimate, and mineral composition that occur across soil horizons, particularly from the organic to the mineral horizons. To test these ideas, we need long-term studies examining how both litter type and the environment of different soil horizons affect initial decomposition, transformation, and potential long-term stabilization of litter-derived SOM (e.g. Voroney et al. 1989; Cotrufo et al. 2015).

In this study, we followed the fate of ^{13}C and ^{15}N labeled needle and fine root litter placed in the O or A soil horizons of a coniferous forest *over 10 years*. During the first five years, more root C and N were retained in the soil than needle C and N (Bird and Torn 2006; Hatton et al. 2015). A larger proportion of the root litter was found in the particulate >2 mm fraction, but similar amounts of root and needle litter were recovered in relatively stable SOM pools (Hatton et al. 2015). However, the influence of soil horizon on decomposition was not yet evident.

The decadal length of our study and the use of isotopic labels allows us to quantify both the loss of litter C and N and its fate as it is transformed into SOM fractions with different turnover times and stabilization mechanisms. The first objective of this study is to investigate how total litter C and N retention over 10 years varies by litter type (needle or root) and soil horizon (O or A). The second objective is to investigate how litter-derived inputs are transformed into SOM and where that SOM is stabilized. We determined the recovery of needle- and fine root-derived C and N in two size fractions (coarse particulate >2 mm and <2 mm soil in the O and A horizon) throughout

10 years and in four density fractions (A horizon only) after 10 years.

Methods

Field study

The study site was a 90 year-old coniferous forest dominated by *Pinus ponderosa* in the Blodgett Experimental Forest outside of Georgetown, CA in the foothills of the Sierra Nevada at 120°38′30″W; 38°53′00″N and 1350 m elevation. Mean annual temperature is 12.5 °C and mean annual precipitation is 1774 mm, most of which falls as snow from November through March (Bird and Torn 2006). Soil temperatures generally remain above freezing and in many years the snow does not remain on the ground for more than a week at a time. The soils are classified as sandy, mixed, mesic Ultic haploxeralfs.

In 2001, labeled *P. ponderosa* needle (3.8 atom % ^{13}C, 5.5 atom % ^{15}N) and fine root (3.3 atom % ^{13}C, 8.1 atom % ^{15}N) litter was inserted into soil mesocosms (10.2 cm diameter by 23 cm deep schedule 40 PVC tube with two 5 cm diameter 450 µm mesh windows opposite one another) that had been driven into the soil to incubate at the field site (Bird and Torn 2006). The treatments were a factorial of litter type (fine roots or needles) and soil horizon (O or A) with $n = 4$. Litter was added to the mesocosms at rates of 147 and 135 g m^{-2} for needles and roots, respectively. Litter was placed within the 2–4 cm depth of the O or the 2–4 cm depth of the A horizon. The O horizon averaged 8 cm deep and about 15 cm of the A horizon was within the mesocosms (Bird and Torn 2006). The mesocosms were collected after 0.5, 1.5 (Bird and Torn 2006; Bird et al. 2008), 5 (Hatton et al. 2015), and 10 years.

Soil processing, fractionation, and analyses

Upon collection, the soil from each mesocosm was separated into the O horizon and 0–10 and 10–15 cm depths of the A horizon. The soil was sieved through a 2 mm mesh into coarse particulate (>2 mm) and bulk soil (<2 mm) size fractions. These size fractions were ground into a fine powder and run on an elemental analyzer for C and N (Costech Model 4010, Valencia, CA) and an isotope ratio mass spectrometer (IRMS, Europa Scientific INTEGRA IRMS, England) for $\delta^{13}C$ and $\delta^{15}N$ at the University of California Davis Stable Isotope Facility.

For the 10 year samples, we separated the A horizon bulk soil into a free light (e.g., fine particulate) and three mineral-associated dense fractions (DF) using a sequential density fractionation based on Sollins et al. (2006, 2009). We added 100 ml of 1.75 mg cm^{-3} of low C/N sodium polytungstate solution (SPT_0, TC-Tungsten Compounds Inc., Grub am Forst, Germany) to 40 g of dried (at 35 °C) soil <2 mm in a centrifuge bottle. The bottle was placed on a shaker table for five minutes on low before being centrifuged in a swinging bucket rotor for 1.5 h at 1929 *g* and 25 °C to ensure no dense particles greater than 0.8 µm remained in the supernatant. The free light fraction was aspirated from the tube, filtered through a 0.8 µm polycarbonate filter (Nucleopore Track-Etch, Whatman), and rinsed with deionized water until the filtrate was the density of water. We collected the FLF by rinsing it off the filter. The solution with the remaining soil in the centrifuge tube was then brought to a density of 2.5 g cm^{-3}, shaken for an hour, and centrifuged (1929 *g*, 25 °C) for 24 h. The fraction that floated (DF1) was aspirated into a new centrifuge tube where it was rinsed with deionized water and centrifuged to allow removal of the supernatant until the supernatant was the density of water. This procedure was repeated with a 2.7 g cm^{-3} density solution (centrifuged for 36 h, 1929 *g*, 25 °C) to separate the remaining soil into the second (DF2) and third (DF3) dense fractions. Average recoveries were 100% for mass, 87% for C, and 83% for N. The densities were chosen based on X-ray diffraction and microscopic inspection of ground samples, which showed the fractions differed in their dominant mineralogy (Table 1; Fig. S1). The X-ray diffraction instrument (PANalytical X'Pert Pro) used Co radiation, an hour long run time per sample, and a continuous scan with a step size of 0.0167. Peaks were identified using X-Pert High Score Plus software.

All fractions were freeze-dried, weighed, ground, and analyzed by EA-IRMS (IsoPrime 100 IRMS in line with a Vario micro cube EA, Isoprime, United Kingdom) for %C, %N, $\delta^{13}C$, and $\delta^{15}N$. Radiocarbon analysis was performed at the Lawrence Livermore National Laboratory Center for Accelerator Mass Spectrometry. Mean residence times (MRT) of the fractions were calculated using a one-pool stock-flow

Table 1 Characteristics of A horizon control soil <2 mm density fractions for the 10 year samples

	Fine particulate	Dense 1	Dense 2	Dense 3
Density (g cm^{-3})	<1.75	1.75–2.50	2.50–2.70	>2.70
Minerals	None	Kaolinite, gibbsite, vermiculite[a]	Quartz, feldspar[a]	Biotite/muscovite[a], Iron oxide coatings[b]
Mass (% of soil)	7 ± 0.6^{A}	23 ± 1^{B}	65 ± 2^{C}	5 ± 0.5^{D}
C (%)	37 ± 0.7^{A}	5.6 ± 0.2^{B}	0.56 ± 0.02^{C}	0.73 ± 0.02^{C}
C (% of soil C)	61 ± 1^{A}	29 ± 1^{B}	8.7 ± 0.6^{C}	0.85 ± 0.1^{D}
N (%)	1.0 ± 0.03^{A}	0.32 ± 0.01^{B}	0.04 ± 0.002^{C}	0.05 ± 0.002^{C}
N (% of soil N)	43 ± 2^{A}	41.5 ± 1^{A}	14 ± 1^{B}	1.5 ± 0.2^{C}
C:N	36.0 ± 1^{A}	17.5 ± 0.4^{B}	15.5 ± 0.5^{BC}	14.3 ± 0.5^{C}
$\delta^{13}C$ (‰)	-26.2 ± 0.15^{A}	-25.1 ± 0.14^{B}	-24.6 ± 0.10^{B}	-24.7 ± 0.15^{B}
$\delta^{15}N$ (‰)	1.7 ± 0.31^{A}	3.6 ± 0.19^{B}	4.5 ± 0.20^{C}	3.8 ± 0.19^{BC}
MRT (years)	5.6 ± 2.4^{A}	127 ± 36^{BC}	100 ± 25^{C}	199 ± 39^{B}

Means are presented with standard error in parentheses (n = 12 for all except stable isotopes, n = 4, and turnover time, n = 3). Letters not shared indicate significant differences among fractions ($\alpha = 0.05$)

[a] Identified using X-ray diffraction

[b] Identified using a microscope

model with a 5 year time lag as described in Bird et al. (2008). For each fraction, only one of the two mathematical solutions was feasible in the context of the fractions' radiocarbon values in 2002 (Bird et al. 2008).

The amount of litter-derived C and N recovered in each fraction was calculated by multiplying the fraction of litter-derived C or N by the total amount of C or N in that fraction. The proportion of litter-derived C or N in each fraction was calculated using a simple mixing model:

$$I_{LS} = f_{soil} \times I_{CS} + f_{litter} \times I_{litter}$$

$$1 = f_{soil} + f_{litter}$$

where I is the isotopic content (^{13}C or ^{15}N), subscripts LS denotes the soil that had labeled litter added to it, CS denotes the control soil, and $litter$ denotes the labeled root or needle litter. f_{soil} is the proportion of C or N from native soil and f_{litter} is the proportion of C or N from the labeled litter. The percent of litter-derived C or N remaining was calculated as the amount of recovered C or N divided by the total amount added to the soil at the beginning of the experiment multiplied by 100.

Statistical analyses

Analyses of variance (ANOVA's) were performed in R (R Development Core Team 2017) on the percent of applied C or N remaining after 10 years with litter type and soil horizon as the main effects plus a litter type by horizon interaction. For the density fractions, fraction was the main effect and, when comparing amounts of litter-derived C and N in the fractions, litter type was an additional main effect. Tukey HSD tests were used post hoc to determine significant differences when a main effect or interaction was significant at the $\alpha = 0.05$ level. Percentages were arcsine square-root transformed prior to analyses to meet statistical assumptions. All residuals were visually inspected for normality and homogeneity of variance.

To quantify the decomposition dynamics of the different litter types in the two horizons, we fit exponential decay models using the bbmle package in R (Bolker 2012). Corrected Akaike's Information Criteria (AIC_c), based on the maximum likelihood, numbers of parameters, and amount of data, were used to select the optimal model (Bolker 2008). We first tested whether a single or double exponential decay curve fit the data better and found the double exponential decay model was best:

$$y = P_f \times e^{-k_f t} + \left(1 - P_f\right) \times e^{-k_s t}$$

where y is the fraction of applied C remaining, P_f is the proportion of the initial C in the fast-decomposing pool, k_f is the decay constant of the fast-decomposing pool, and k_s is the decay constant of the slow-

decomposing pool. We tested whether these three variables needed to vary by litter or horizon in order to best fit the data. With 3 variables, each of which could be affected by none, one, or both factors, there were 28 candidate models to test. To reduce the number of candidate models, we first optimized the model structure for *P,* because the AICc was most sensitive to *P,* by testing *P* with none, one, or both factors. We tested *P* with both the full (other variables dependent on both litter and horizon) and base (other variables not dependent on litter or horizon) models, and found it did not change the optimal model structure for *P*. We then tested the optimal structure for k_f with the optimal *P* structure and with both full and base model parameterization for k_s. Finally, we tested the optimal model structure for k_s using the optimal *P* and k_f structure. To show the results of the optimal k_s structure did not depend on k_f, we also tested it with the base model parameterization for k_f. We report the parameter estimates for the best model and include 95% confidence intervals based on the likelihood profiles.

Results

Total C and N remaining

After 10 years of decomposition, the total amount of litter-derived C and N remaining in soil mesocosms was not affected by soil horizon placement, but was significantly affected by litter type. While 44% of root C remained, only 22–28% of needle C remained (Table 2; Fig. 1; Table S1). For litter N, 10–15% remained from roots but only 5–6% from needles. The figures show the amount of litter-derived C; litter-derived N followed a similar pattern (Table S1).

The best-fit model to represent C loss over 10 years was a 2-pool exponential decay model, (Table S2) where in the fast-decaying pool, *P,* varied by litter type but not horizon. Over 50% of the needle C and only 22% of root C was in this fast-decomposing pool (Table 3). The best-fit model's k_f varied by both litter type and soil horizon. The value of k_f was greater for needles in the O and A horizon than for roots in the A horizon. The roots in the O horizon had the greatest k_f. However, the model had trouble converging on this value because the roots in the O horizon lost 25% of their C quickly and then loss slowed abruptly. This pattern differs from the more gradual shift in C loss of the other treatments (Fig. 1). While it is clear that the initial phase of root decomposition in the O horizon differed from the other treatments, finer temporal sampling during that phase may have allowed for a better estimate of $k_{f\ root,\ O}$. Lastly, k_s did not differ by litter type or horizon in the best-fit model and was two orders of magnitude slower than k_f (Table 3).

Retention of C and N within soil fractions

Unlike for retention of total C and N, both soil horizon and litter type influenced the partitioning of C and N within soil size fractions after 10 years. We first considered the retention of C and N in the coarse particulate fraction (>2 mm), which is the pool that the labeled litter started in. There was significantly more litter-derived C and N remaining in the coarse particulate fraction for litter placed in the O horizon than in the A horizon and more root-derived C than needle-derived C retained independent of horizon (Table 2, Fig. 2a). In contrast, in the finer <2 mm bulk soil significantly more litter-derived C and N remained for litter placed in the A horizon than litters placed in the O horizon. The amount of litter-derived C and N retained in the bulk soil did not differ by litter type for either horizon (Table 2; Fig. 2b). Across time, the amount of litter-derived C and N retained in the bulk soil declined in the O horizon after two years, but had not declined in the A horizon after 10 years (Fig. 2b).

For the 10 year soil samples, the bulk soil of the A horizon was further separated into one fine particulate (free light) and three mineral-associated dense fractions (Table 1) to investigate stabilization patterns for litter-derived C and N. More root-derived than needle-derived C and N were found in the fine particulate fraction after 10 years (Table 2; Fig. 3). In contrast, litter type did not affect the amount of litter-derived C and N found in the dense fractions (Table 2; Fig. 3). Similar to the distribution of native SOM among fractions (Table 1), most of the remaining litter-derived C and N were found in the fine particulate fraction (Fig. 3; Tables S1, S2).

The initial C:N ratios of the labeled root and needle litter were 49 and 39 (Bird and Torn 2006), respectively, and the C:N ratios of litter-derived OM

Table 2 ANOVA results testing significant effects of litter type and soil horizon on the percent of applied carbon (C) or nitrogen (N) remaining in various fractions 10 years after litter placement

	Total		Particulate > 2 mm		Bulk < 2 mm		Fine Particulate (A only)		Dense (A only)	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
% Applied C										
Litter	14	**0.003**	13	**0.003**	2.1	0.17	5.6	**0.056**	0.29	0.61
Horizon	0.41	0.53	11	**0.007**	16	**0.002**	–	–	–	–
Litter:Horizon	0.32	0.58	2.0	0.18	0.36	0.56	–	–	–	–
% Applied N										
Litter	7.4	**0.018**	3.4	0.09	2.2	0.16	5.0	0.066	0.57	0.48
Horizon	0.53	0.48	17	**0.001**	26	**<0.001**	–	–	–	–
Litter:Horizon	0.0	0.99	0.59	0.36	0.93	0.35	–	–	–	–
C:N										
Litter			100	**<0.001**	21	**<0.001**	4.8	0.072	0.79	0.41
Horizon			12	**0.004**	0.28	0.60	–	–	–	–
Litter:Horizon			2.0	0.18	0.85	0.37	–	–	–	–

The degrees of freedom were 1 for all effects. Bold text indicates significance ($\alpha = 0.05$)

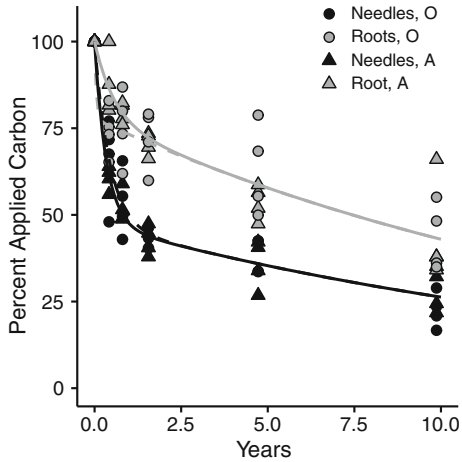

Fig. 1 The percent of applied carbon remaining over 10 years in a coniferous forest soil. The *black symbols* are needle litter and the *gray symbols* are root litter. *Circles* are the litter that was placed in the O horizon, and *triangles* are the litter that was placed in the A horizon. The *line* shows the predicted values from the optimal double exponential decay model; the *dashed line* is for the O horizon and the *solid line* is for the A horizon

Table 3 Estimated parameter values of the best-fit double exponential decay model with their standard error and 95% confidence intervals

Parameter	Value	SE	2.5%	97.5%	p-Value
$P_{f\ needle}$	0.52	0.023	0.48	0.57	<0.0001
$P_{f\ root}$	0.22	0.021	0.18	0.26	<0.0001
$k_{f\ needle,\ A}$	2.95	0.56	2.1	4.6	<0.0001
$k_{f\ needle,\ O}$	2.35	0.39	1.7	3.4	<0.0001
$k_{f\ root,\ A}$	1.76	0.54	1.0	3.7	0.001
$k_{f\ root,\ O}$	20.1	NA	NA	NA	<0.0001
k_s	0.059	0.0057	0.048	0.078	<0.0001

Standard error and confidence intervals for $k_{f\ root,\ O}$ were unavailable (see Results)

declined over time in the >2 mm and <2 mm soil fractions (Fig. 2). After 10 years of decomposition, root-derived OM had a significantly higher C:N ratio than needle-derived OM in both size fractions and in both horizons (Table 2; Fig. 2). The C:N ratio of litter-derived OM was significantly lower in the dense fractions than in the fine particulate fraction; a pattern similar to native SOM C:N in the fractions, except the litter-derived C:N ratio did not differ among the dense fractions (Fig. 3; Table 1).

Vertical transport of C and N

During the 10 years after placement, very little of the applied C was transported and retained in soil at lower depths. The amount of applied C and N transported from the O horizon and recovered in the top 10 cm of the A horizon was 1.7 ± 0.5% and 7.4 ± 1.1% for

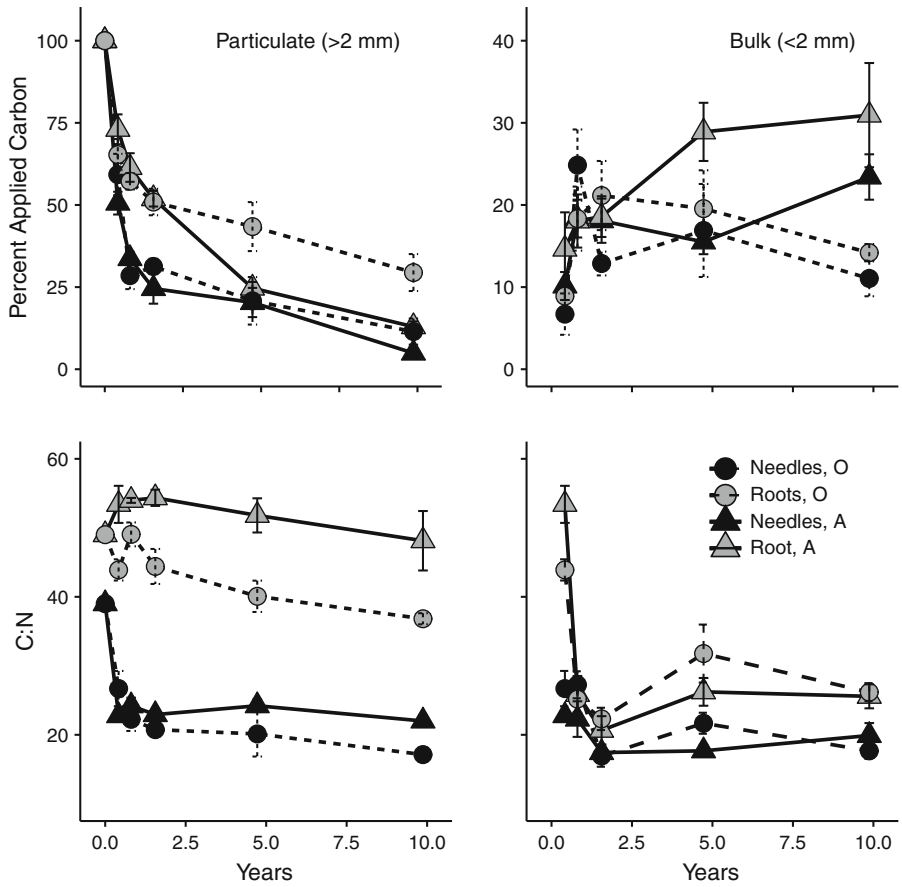

Fig. 2 The percent of applied carbon remaining (*top graphs*) and the C:N of litter-derived organic matter remaining (*bottom graphs*) in the coarse particulate (>2 mm) and bulk soil (<2 mm) fractions of the O and A horizons over 10 years of decomposition. O horizon are represented by *circles* and *dashed lines* and the A horizon are represented by *triangles* and *solid lines*; *gray symbols* are root litter and *black symbols* are needle litter. *Error bars* represent the standard error (n = 4)

needles and 3.4 ± 1.1% and 9.1 ± 2.2% for roots (Table S3). The amount of applied C and N transported from the A horizon 0-10 cm depth and recovered in the A horizon 10-15 cm depth was 1.5 ± 1.3% and 4.1 ± 1.6% for needles and 1.0 ± 0.5% and 3.9 ± 1.7% for roots, (Table S3). Less than 1.5% of C and less than 2.1% of N was transported upwards from the A and retained in the O horizon (Table S3). There were no significant differences in the amount of applied C and N transported and retained among litter type ($p = 0.16$), but more N than C was transported and retained ($p < 0.001$).

Discussion

We followed the fate of two contrasting plant inputs, needles and fine roots, in two contrasting soil horizons, organic and mineral, for 10 years. The controls on decomposition and stabilization shifted over time. Litter chemistry and morphology governed initial C and N losses, with needles decomposing faster than roots initially, regardless of soil horizon. However, the influence of the soil environment grew over time. Physical breakdown (to <2 mm) of new inputs was faster in the A horizon than in the O horizon, but once physically broken down, further decay was slower in the A horizon, due to the organo-mineral associations available in that horizon. Considering potential long-term stabilization, initial differences in decomposition between litter types had little effect. By 10 years, needle and root-derived C (and N) were equally likely to be complexed with minerals. Determined for the first time directly, the efficiency of stabilization (the fraction of inputs transformed to a physico-chemical state with long turnover times) was about 4.6% of initial C inputs stabilized on minerals and 16% for N. We discuss litter-derived C and N results together because their retention patterns were quite similar. However, similar patterns do not imply they cycled

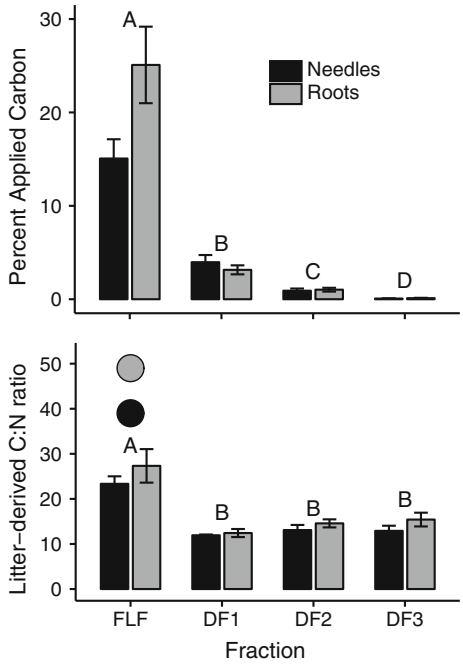

Fig. 3 The percent of applied root (*gray*) and needle (*black*) carbon in the density fractions of the A horizon after 10 years. The *circles* represent the initial C:N of the litter. *Error bars* represent the standard error (n = 4). *Letters* not shared indicate significant differences ($\alpha = 0.05$). There was significantly more root litter remaining in the free light fraction as a percent of applied (*FLF* litter × fraction, f = 4.8, p = 0.009). The FLF has a significantly greater C:N ratio than do the dense fractions ($p < 0.0001$)

within the soil in tandem as evidenced by the greater vertical mobility of N.

The effect of litter type

The most important factor determining total C and N soil retention after a decade was the litter type. Initial C and N losses (0–18 months) were much faster for needles than for fine roots (Bird and Torn 2006). This corroborates findings from numerous litter bag-type decomposition studies that needles decompose faster than fine roots (Hansson et al. 2010; Freschet et al. 2013). The main decomposition differences between litter types was the proportion of C in fast-decomposing pool (C_f), which was 52% of the total needle C, but only 22% of the total root C, and the decomposition rates of that pool (k_f). The differences in C and N loss after 10 years appeared to be a legacy of these initial dynamics because the long-term C loss rates (based on k_s) were similar for both litter types and soil horizons. Thus, total litter-derived C retention in soil, even after a decade, was driven by intrinsic traits that affect the fast-cycling pool size and its decay, like tissue chemistry (e.g. more soluble carbohydrates in needles than leaves; Bird and Torn 2006; Mambelli et al. 2011), anatomy (e.g. root's casparian strip; Hose et al. 2001), and their interaction.

The effect of soil horizon placement

In contrast, the soil horizon the litter was placed in did not affect the total C and N retention. This finding corroborates several shorter-term (12–36 month) studies that did not find decomposition differences in a common substrate among soil horizons (Fujii and Takeda 2010) or depths (Sanaullah et al. 2011; Solly et al. 2015). Similarly, traditional litter decomposition studies have found that within an ecosystem or among similar ecosystems, litter type explains most of the variation in mass loss (e.g. Aerts 1997; Dorrepaal et al. 2005).

Soil horizon, however, did have an important long-term influence on decomposition processes. The mineral component of the O horizon (34% C), was very low compared with the A horizon (5.7% C), rendering protection by organo-mineral associations relatively insignificant. As a result, the amount of litter-derived C and N retained in the O horizon bulk soil began to decrease after two years while the amount of C and N retained in the A horizon bulk soil was steady over 10 years. Despite the lack of mineral protection, the O horizon retained 22% of needle C and 44% of root C after a decade.

The persistence of C and N from both litter types in the O horizon, particularly of root-derived coarse particulate C, implies that mechanisms other than physicochemical protection retard decomposition (Marschner et al. 2008; Dungait et al. 2012). The lack of bioturbation and cryoturbation in this ecosystem, which limits fragmentation of litter, allows a thick O horizon (8–10 cm depth) to develop. This lack of turbation likely interacted with root litter's anatomical and chemical structure, whereby outer layers of suberin and cutin retard enzymatic, pathogenic, and faunal access to roots thereby slowing the disintegration and transformation of root litter into the smaller size fractions. In addition, the temperature and moisture extremes of the O horizon (Bird and Torn 2006) can reduce microbial and faunal decay of litter

particles. Particularly, the abundance of soil macrofauna, which are key to litter comminution, may be reduced in the O horizon during the dry seasons (Petersen and Luxton 1982). Although there were large microclimate differences between the O and A horizons (Bird and Torn 2006), the amount of total litter-derived C and N retained has been similar in both horizons.

More litter-derived C and N were retained in the bulk soil of A horizon than in the bulk soil of the O horizon. In the A horizon, this size fraction was separated into a fine particulate and three mineral-associated fractions. After 10 years, the fine particulate fraction was where the majority of the remaining OM from both litter types was retained, but more OM from roots than from needles was retained as fine particulates. Previously, there was more needle-derived OM in this pool after 1.5 years (Bird et al. 2008), and the same amount for both needle- and root-derived OM after 5 years (Hatton et al. 2015). This slow build up of root material as fine particulates was likely due to the roots' chemical recalcitrance (Bird and Torn 2006) and morphology, which retarded the transformation of root litter out of the particulate and into this finer size fraction.

Organic matter in mineral complexes has been transformed from the original plant inputs. This OM includes microbial by-products (Baisden et al. 2002; Gleixner 2013; Hatton et al. 2014) and leached small molecular weight dissolved OM, both of which can have narrower C:N ratios than the original plant material. It has been hypothesized that once transformed through the "microbial-DOM" pathway (Cotrufo et al. 2015), the structural and molecular origins of C and N will no longer influence its retention, but this concept has not been tested beyond initial decay stages. We found that the amount of litter-derived OM retained in organo-mineral associations was the same for both types of plant inputs after 10 years and that the ^{13}C label had been incorporated into all compound classes of the bulk soil regardless of litter type (Mambelli et al. 2011). Furthermore, the decadal-scale efficiency of this transformation and complexation has not been measured previously, but here we were able to determine that 4.6% of the litter-derived C and 16% of the N were in organo-mineral associations 10 years after placement in the A horizon.

In many soils, organo-mineral associations appear responsible for the long residence times of SOM (Torn et al. 1997; Mathieu et al. 2015). Consistent with those findings, the average residence time of C in the fine particulate fraction was only 5 years at our site, while in the dense fractions it ranged from 100 to 200 years at only 0–10 cm depth (Table 1; Table S4) likely due to the mineral protection of OM in the dense fractions that limits microbial access (Lehmann and Kleber 2015). Each dense fraction isolated in our study was dominated by different minerals, with different reactivities and modes of OM stabilization. Similar to other sequential density fractionations (Baisden et al. 2002; Sollins et al. 2006, 2009), the narrowing C:N ratio indicated that the extent of SOM decay or the affinity for N-containing compounds increased with the fraction density. The densest fraction, containing iron oxides and primary phyllosilicate minerals had the lowest native soil C:N ratio and the longest MRT. However, only 0.09% of the applied litter-derived C and 0.29% of litter-derived N (about 0.35 and 0.55% of remaining litter-derived C and N, respectively) were in this fraction after 10 years. About 0.85 and 1.5% of native soil C and N were found in this fraction. Generally, the litter-derived OM follows the distribution of the native SOM amongst the dense mineral fractions, with the most being found in the least dense fraction and the least in the densest fraction. However, the litter-derived OM has similar C:N across all mineral fractions while the native SOM has decreasing C:N, which implies that it will take longer than 10 years for the cycle of organo-mineral association and microbial processing of the labeled litter to reach steady state.

The relatively N-rich compounds and long turnover times in the densest fraction suggest that direct interactions between the minerals and peptide-containing compounds, which likely derive from microbial by-products (Mambelli et al. 2011), are the likely stabilization mechanisms (Sollins et al. 2006). The secondary clay minerals and high percentage of native C in the least dense mineral fraction imply OM there may be stabilized within aggregates (Hatton et al. 2012). In the intermediate density mineral fraction, the low reactivity of the quartz and feldspar suggest an association with minerals that is mediated by adhesive microbial metabolites or microbial cells (Hatton et al. 2012).

In one conceptual model for the transformation of plant litter to SOM, MEMS, labile litter is thought to be the dominant source of stable SOM because it is

more efficiently processed through microbes after which it can become protected by minerals (Cotrufo et al. 2013; Haddix et al. 2016). If so, more needle-derived than root-derived OM would be associated with minerals because the needles had a larger fast-decaying C pool. Initially, we found more needle-derived OM relative to root-derived OM in microbial biomass (Bird and Torn 2006) and in mineral-associated SOM fractions (Bird et al. 2008; Hatton et al. 2015). However, after ten years, the same amount of root- and needle-derived OM was associated with minerals. While needle inputs did move to mineral-complexes more quickly, we saw no evidence that initial lability enhanced the amount of litter-derived SOM stabilized in the mineral matrix in the long term. While our results may contradict MEMS, the lack of difference between litter types could also be a result of soil C saturation (Castellano et al. 2015), and the soil C saturation status of our soils is unknown. If our soils are not C saturated, it is possible that more root than needle C and N will become mineral-associated after the roots' slower transition out of the coarse and fine particulate pools.

Implications for long-term SOM storage

Based on our study, we can estimate the amount of C and N from needle and root production that becomes stored in mineral-associated soil pools with at least centennial-scale mean residence times. At Blodgett forest, annual litterfall is 474 g C m^{-2} y^{-1}, of which $\sim$40% (206 g Cm^{-2} y^{-1}) is needles (Stohlgren 1988) and annual fine root production is 38-45 g C m^{-2} y^{-1} (Gaudinski et al. 2010). Using the initial litter C:N (Bird and Torn 2006), this equates to an input of 5.3 and 0.8-0.9 g N m^{-2} y^{-1} from needles and roots, respectively. In the A horizon, roughly 4.3% or 1.6–1.9 g C m^{-2} y^{-1} (and 17% or 0.14 g N m^{-2} y^{-1}) of root input is found in the mineral-associated pool after 10 years. A small proportion of needle fall C and N will be incorporated into the mineral soil; only about 2% (4.1 g C m^{-2} y^{-1}) of needle C and 9% (0.48 g N g C m^{-2} y^{-1}) of needle N from the O horizon was transported to and retained in the A horizon after 10 years. We assume this transported material has become mineral-associated because its low C:N ($\approx$8) matches that of dense fraction native SOM. Despite low transport efficiency, needles may be a larger source of stabilized (mineral-associated) OM in the A horizon than roots due to greater production rates. For deeper horizons, needle input is likely less important, since there was only 20% as much O-horizon input retained at 10–15 cm as at 0–10 cm. As a percentage of production, more root OM (4.3%) than needle OM (2%) becomes associated with A horizon minerals; thus, on a per unit production basis, increasing root C allocation would increase potential long-term soil C storage more than increasing needle production.

The importance of long term studies

Few studies have examined decomposition processes from its initial stages to SOM formation, transport, and stabilization over a decade. We used our unusual perspective to examine how decomposition dynamics change over time. Some of the patterns observed after 1.5 years persisted through the decade. For example, litter-derived C and N losses were initially faster for needles than for roots and thus, total retention of C and N remained higher for roots. However, the difference in loss rates was transient; decomposition rates for the slow-decaying pool of each litter were the same. Moreover, retention in mineral-associated fractions was the same for roots and needles. Most significantly, it was only after 10 years that the influence of soil horizon on the fate and amount of litter-derived C and N retained became evident: more litter-derived C and N were retained in the coarse particulate fraction of the O horizon due to conditions that retard physical fragmentation and in the bulk soil of the A horizon due to protective mineral associations.

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