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Incorporation of shoot versus root-derived ^{13}C and ^{15}N into mineral-associated organic matter fractions: results of a soil slurry incubation with dual-labelled plant material

J. M. Lavallee · **R. T. Conant** · **E. A. Paul** · **M. F. Cotrufo**

Abstract Mineral-associated organic matter (MAOM) is a key component of the global carbon (C) and nitrogen (N) cycles, but the processes controlling its formation from plant litter are not well understood. Recent evidence suggests that more MAOM will form from higher quality litters (e.g., those with lower C/N ratios and lower lignocellulose indices), than lower quality litters. Shoots and roots of the same non-woody plant can provide good examples of high and low quality litters, respectively, yet previous work tends to show a majority of soil organic matter is root-derived. We investigated the effect of litter quality on MAOM formation from shoots versus roots using a litter-soil slurry incubation of isotopically labeled (^{13}C and ^{15}N) shoots or roots of Big Bluestem (*Andropogon gerardii*) with isolated silt or clay soil fractions. The slurry method minimized the influence of soil structure and maximized contact between plant material and soil. We tracked the contribution of shoot- and root-derived C and N to newly formed MAOM over 60 days. We found that shoots contributed more C and N to MAOM than roots. The formation of shoot-derived MAOM was also more efficient, meaning that less CO_2 was respired per unit MAOM formed. We suggest that these results are driven by initial differences in litter chemistry between the shoot and root material, while results of studies showing a majority of soil organic matter is root-derived may be driven by alternate mechanisms, such as proximity of roots to mineral

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surfaces, greater contribution of roots to aggregate formation, and root exudation. Across all treatments, newly formed MAOM had a low C/N ratio compared to the parent plant material, which supports the idea that microbial processing of litter is a key pathway of MAOM formation.

Keywords Soil organic matter · Decomposition · Litter quality · Mineral-associated organic matter · Soil incubation · Microbial respiration

Introduction

Soil organic matter (SOM) contains enormous stocks of carbon (C) and nitrogen (N) (Batjes 2014), is a major source and sink of atmospheric greenhouse gases (Leifeld 2006), and plays a key role in soil health and fertility (Smith et al. 2015). Mineral-associated organic matter (MAOM) is the largest pool of soil organic C and N, and often contains the oldest C in a given ecosystem (Anderson and Paul 1984; Christensen 2001; Mikutta et al. 2006; Kögel-Knabner et al. 2008). Because the formation of MAOM has the potential to form a negative feedback to climate change and provide long-term improvements to soil quality, interest in the controls on MAOM formation has increased considerably in recent years (Cotrufo et al. 2013; Lehmann and Kleber 2015).

Two key controls on MAOM formation are plant litter chemistry (both initial differences in litter chemistry, and changes in litter chemistry over the course of decomposition), and soil texture. Litter chemistry can control MAOM formation through two mechanisms: directly, through the amount and type of soluble plant compounds that may leach from the litter (Klotzbücher et al. 2011; Soong et al. 2015) and interact directly with mineral surfaces or other MAOM without microbial transformation (see Kramer et al. 2012); or indirectly, by affecting the efficiency with which microorganisms use the litter to produce microbial products, which are precursors to MAOM (Sanderman et al. 2014; Kallenbach et al. 2016; Paul 2016). According to the Microbial Efficiency-Matrix Stabilization (MEMS) framework from Cotrufo et al. (2013), plant litter that is of higher initial quality—commonly defined as containing a higher ratio of labile-to-recalcitrant compounds and a lower

C-to-nutrient ratio—may be utilized more efficiently by microorganisms, leading to greater formation of MAOM and lower production of CO_2 in soils with available capacity for MAOM storage (Cotrufo et al. 2013). This idea can also apply to the same plant litter at different points during decomposition (Cotrufo et al. 2015). MAOM formation by this microbial mechanism may be more efficient early on in decomposition, when more labile and soluble compounds are present, than later on when those compounds have been decomposed and what remains is relatively less labile.

The texture of the soil is also a control on MAOM formation, as it affects the total area and chemistry of the surfaces that OM associates with (Torn et al. 1997; Wattel-Koekkoek et al. 2001; Feng et al. 2005; Kögel-Knabner et al. 2008; Sanderman et al. 2014) and also the rate at which MAOM forms (Six et al. 2002; Stewart et al. 2007). Hassink et al. (1997) and Six et al. (2002) found close positive correlations between the percent of soil mass in the silt + clay fraction, and the amount of C in that same fraction (which we refer to as MAOM-C). These correlations can be used as a method to estimate the capacity of a soil to stabilize C, also known as a soil's "saturation deficit" (Stewart et al. 2007). When the saturation deficit is larger, the rate of storage by the silt and clay is faster, and as the saturation deficit shrinks, the rate of storage slows. Castellano et al. (2015) combined the ideas behind soil C saturation and the MEMS framework to posit that the effect of litter quality on MAOM formation is a function of the saturation deficit. They hypothesize that if the saturation deficit is high, there will be little or no effect of litter quality on MAOM formation, because the rate of storage will be high regardless of litter quality. As the saturation deficit decreases, the effect of litter quality on the rate of MAOM formation will become clearer, because the rate of formation of MAOM becomes less dependent on the saturation deficit, and more dependent on litter quality. However, as long as the soils have not yet reached saturation, soils receiving high quality litters will have more MAOM than soils receiving low quality litters (Castellano et al. 2015).

One common way to study interactions of soil texture and MAOM is by physical separation of silt- and clay-sized soil fractions (Christensen 2001). Silt and clay organo-mineral particles (2–53 μm and < 2 μm, respectively) account for the majority of MAOM in soils (Anderson and Paul 1984;

Christensen 2001; Kögel-Knabner et al. 2008), but the two fractions have different association capacities (Balesdent et al. 1996; Stewart et al. 2008), and the SOM associated with them has different chemical properties (Balesdent et al. 1987; Plante et al. 2006; Grandy and Neff 2008; Calderón et al. 2011). Given that silt and clay may saturate at different rates, and that saturation can determine the effect of litter quality on MAOM formation (Castellano et al. 2015), we sought to investigate the interactive effects of litter chemistry and soil texture on MAOM formation during different phases of litter decomposition.

In this study, we performed a two-factorial (litter type and silt or clay) soil slurry incubation using isotopically labeled (^{13}C and ^{15}N) roots and shoots of *Andropogon gerardii*, a grass species common to tallgrass prairie in the United States. Grass roots and shoots provide a good opportunity to explore the role that initial litter quality plays in the formation of C and N in MAOM because they come from the same plant grown under the same conditions, but roots tend to have higher contents of recalcitrant compounds such as lignin and suberin (Rasse et al. 2005; Abiven et al. 2005). Previous work shows that root material contributes more to SOM than shoot material, even when differences in biomass production are accounted for (Rasse et al. 2005). But the majority of studies comparing root and shoot-derived SOM have not specifically examined MAOM, and include several different mechanisms of SOM formation operating simultaneously [e.g., particulate organic matter (POM) formation and aggregation (Tisdall and Oades 1982; Cambardella and Elliott 1992; Six et al. 1999; Paul 2016)].

A deeper understanding of the mechanisms of SOM formation from roots and shoots is needed, and this requires separating the multiple mechanisms that may confound results. We used a litter-soil-slurry technique (Wallenstein et al. 2012) to minimize some of those competing mechanisms—namely aggregation and spatial separation of decomposers, substrates, and mineral surfaces—and focus on litter type as a driver of microbial decomposition and subsequent association of litter-derived C and N with silt and clay particles. Constant shaking in water minimized aggregate formation and promoted equal contact between organic material and the mineral matrix for both shoots and roots. We investigated MAOM formation during two phases of litter decomposition, the "short

term" and the "mid term". In the short term, non-structural water-soluble compounds leach from litter and are processed by microorganisms with very high efficiency (Cotrufo et al. 2015). Once this initial leaching has stopped, decomposition enters the mid term, when microorganisms decompose cellulose and other insoluble litter compounds (Soong et al. 2015). This mid term litter processing is less efficient than in the short term, according to general theory (Manzoni et al. 2012; Sinsabaugh et al. 2013). We studied the short term by harvesting a set of samples very early in the incubation, just after the initial flush of CO_2 had ended (at day 7). We harvested a second set of samples after 60 days of incubation to study the mid term.

We predicted that, if shoots were of higher initial quality than roots, then: (1) more MAOM would form from shoots than from roots, (2) MAOM formation from shoots would be more efficient (more MAOM formed and less CO_2 produced per unit of litter processed) than from roots during both phases of decomposition, and (3) in both litters, MAOM formation would be more efficient in the short term than in the mid term of litter decomposition.

Methods

Growth of isotopically labeled plant litter

Big bluestem (*Andropogon gerardii*) was grown from seedling to maturity in a continuous, isotopic labeling chamber at Colorado State University (Soong et al. 2014). This procedure yielded shoots that were 4.7 atom% [13]C and 6.5 atom% [15]N, and roots that were 4.5 atom% [13]C and 6.5 atom% [15]N. When plants reached early senescence, aboveground biomass (which we refer to as shoots) was clipped, and belowground biomass (which we refer to as roots) was separated from the growth medium (a mixture of sand, vermiculite, and profile porous ceramic), and both were air-dried. Random subsamples of the dried shoots and roots were clipped to 0.5–1.5 cm in length, and any pieces that passed through an 800 μm-mesh sieve were discarded. The aim of sieving was to obtain pieces of plant litter that could be readily separated from the silt or clay using a 250 μm sieve after the incubation. Subsets of root and shoot litter were further oven-dried at 60 °C, ground, and analyzed for δ^{13}C, δ^{15}N, and C and N concentrations on a Costech

ECS 4010 (Costech Analytical Technologies, Valencia, CA USA) coupled to a Delta V Advantage isotope ratio mass spectrometer (IRMS) (Thermo-Fisher, Bremen, Germany).

Chemical characterization of shoots and roots

Hot water extractions were performed on four replicates each of shoots and roots using a procedure modified from Haddix et al. (2016). Briefly, 0.7 g of air-dried shoots or roots were combined with 40 ml of hot, deionized water in a covered test tube and kept in a digestion block at 100 °C for 3 h. Samples were then poured over a 20 μm nylon filter to separate extracted material from the residue. Extracts were analyzed for organic C and N using a TOC analyzer (Shimadzu TOC-L, Shimadzu Scientific Instruments, Inc.).

We performed wet chemical fractionation of shoots and roots using the acid detergent fiber (ADF) digestion method (Goering and Van Soest 1970). Subsamples (0.3 g) of shoots and roots were first digested in cetyl trimethylammonium bromide (CTAB) and sulfuric acid to remove hemicellulose and other non-structural carbohydrates and lipids. Next, they were digested in 73% sulfuric acid to remove the acid hydrolyzable fiber (AHF), which can be used as a proxy for cellulose (McKee et al. 2016). The sulfuric acid digestion left behind the acid-unhydrolyzable residue (AUR). Both the AHF and AUR fractions were adjusted for ash content. Lignocellulose index was calculated as [AUR/(AUR + AHF)] (Soong et al. 2015). Residues of the hot water extraction and ADF digestion were dried overnight at 105 °C prior to weighing. Previous studies have shown that the AUR is made up primarily of lignin, but may also contain significant proportions of plant waxes, cutin, suberin, and condensed tannins (Preston and Trofymow 2015). We considered litter with a lower C/N ratio, higher proportion of hot water extractable C and N, lower lignocellulose index, and lower AUR/N ratio to be of higher initial quality.

Fractionation of silt and clay

The soil used in this experiment was collected from cultivated wheat fields at Waggoner Ranch in northern Texas, south of the town Vernon in Wilbarger County (33°50′N, 99°02′W) for a previous study by Haddix et al. (2011). The soil was collected from 0 to 20 cm

after removal of aboveground vegetation and surface litter. It is a shallow, smectitic, Vernon Series clay loam (Martin et al. 2003). This soil was chosen for its high silt and clay content (approx. 80% silt + clay) and low SOM content (1.02 ± 0.04) (Haddix et al. 2011), under the assumption that it would have a large capacity to stabilize additional MAOM. Silt and clay were isolated from the whole soil by a physical fractionation scheme modified from Jagadamma et al. (2013). Briefly, oven dried (60 °C) bulk soil was dispersed by shaking for 18 h in DI water with glass beads. After dispersion, the soil and water mixture was poured over a 53 μm mesh screen and gently sieved to remove particulate organic matter and other sand-sized material. We chose not to disperse using chemical techniques because chemical dispersants can denature enzymes and interfere with microbial activity (Allison and Jastrow 2006; Jagadamma et al. 2013). Similarly, we chose not to employ a density separation because high density liquids such as SPT can reduce microbial activity (Crow et al. 2007; Jagadamma et al. 2013). After sieving, the < 53 μm material was sonicated to further disperse microaggregates. We chose the energy of sonication (720 J cm^{-3}) based on preliminary testing which showed the maximum amount of clay that could be produced from dispersion of silt-sized aggregates by sonication for this soil. After sonication, silt and clay were separated by centrifugation at 20 °C according to Stokes' Law, and oven dried at 105 °C. The high drying temperature of 105 °C was chosen because silt and clay fractions retained water after drying at 60 °C. It is likely to have negatively affected the soil microbial population, but subsequent tests revealed significant microbial activity when the dried soil fractions were rewetted. Dried silt and clay were gently broken up and homogenized using a mortar and pestle. Subsamples were then taken, and four replicates of each were analyzed for δ^{13}C, δ^{15}N, C and N concentrations on a Costech ECS 4010 (Costech Analytical Technologies, Valencia, CA USA) coupled to a Delta V Advantage IRMS (Thermo-Fisher, Bremen, Germany).

Specific surface area (SSA) of the silt and clay fractions were measured using the multi-point Brunauer–Emmett–Teller (BET) method on a Micrometritics Tristar II Series Analyzer (Micromeritics, Norcross, GA) at the University of Minnesota. SSA measurements were made before and after SOM

removal, which was achieved by muffling for 18 h at 350 °C. Muffling at high temperatures can alter soil mineralogy, but all methods for removing organic matter have drawbacks (see discussion in Appendix 1.7.2 of Fisher 2016), so we chose to employ muffling and to interpret the results with caution. Iron and aluminum oxides and oxyhydroxides are thought to play important roles in formation and persistence of MAOM (Mikutta et al. 2006); these can be measured by acid ammonium oxalate extraction, which gives an estimate of amorphous active Fe (Fe_o) and Al (Al_o), and dithionite-citrate extraction, which gives and estimate of the combined content of amorphous forms of Fe and crystalline Fe oxides (Fe_d). The acid ammonium oxalate extraction was performed according to McKeague and Day (1966). Briefly, 0.25 g of silt or clay was mixed with 25 ml ammonium oxalate and oxalic acid (0.2 M) at pH 3 in 50 ml conical-bottom centrifuge tubes. These were shaken in the dark for 4 h, and then centrifuged at 1520 g for 15 min. The dithionite-citrate extraction was performed according to Mehra and Jackson (1960). Briefly, 0.25 g of silt or clay was mixed with 25 ml sodium citrate (0.68 M) in 50 ml conical-bottom centrifuge tubes, and 0.5 g of dithionite was added. The mixtures were shaken for 16 h, and then centrifuged at 1520 g for 15 min. Concentrations of Fe and Al in all extract solutions were measured using inductively coupled plasma optical emission spectroscopy (PerkinElmer Inc., Waltham, MA).

Experimental design and setup

We incubated slurries of silt or clay with roots or shoots in a full factorial design with four replicates. We maintained controls, which included silt or clay that did not receive plant litter (mineral controls), and shoots or roots with no soil (litter controls), also with four replicates. Together, the litter-amended mineral slurries, mineral controls, and litter controls (32 total) constituted one set. We incubated two sets, allowing for one destructive harvest partway through the incubation and another at the end. The first harvest, at day 7, was timed to coincide with the sharp decrease in respiration rates early on—a common characteristic of soil incubations (Voroney et al. 1989; Birge et al. 2015), especially after rewetting (Birch 1958)—and with the period of maximum loss of water-soluble constituents from litter (Soong et al. 2015). The aim

was to capture the dynamics within the "short term" of decomposition, when water-solubles are lost, respiration rates are high, and decomposition is rapid. The second set of samples was harvested after 60 days.

Slurries consisted of 1 g of either silt or clay, 0.1 g of shoots or roots, and 20 ml of deionized water in 50 ml conical-bottom centrifuge tubes with plug seal caps fitted with rubber septa. Following the procedure of Wallenstein et al. (2012), the slurry components were combined on day zero of the incubation, the tubes were capped (airtight), and all samples were flushed with CO_2-free air for 10 min. Samples were then placed on a horizontal shaker at 25 °C and shaken constantly to keep the slurries aerated.

CO_2 flux measurements

CO_2 concentrations were measured on the set of samples that were harvested at day 60, by mixing the headspace with a syringe, taking a 1-ml subsample, and injecting it manually into an LI-6525 infrared gas analyzer (LI-COR, Lincoln, NE). A five-point calibration curve using a standard gas of known CO_2 concentration was used to determine the CO_2 concentration of the samples. CO_2 measurements were taken daily for the first seven days, and every 2–3 days until day 58. On a subset of those days (3, 5, 8, 10, 19, 27, 34, 41, 48, and 58), immediately after CO_2 measurement, the $\delta^{13}C$ of the CO_2 was measured on the same subset of samples using a VG Optima IRMS with a microgas injector and equilibration block (Isoprime Inc., Manchester, UK). Gas samples for $\delta^{13}C$ analysis were taken directly from slurry tubes using a syringe, and injected into the IRMS without intermediate storage. For days when $\delta^{13}C$-CO_2 was not measured, the $\delta^{13}C$-CO_2 was estimated using linear interpolation between the prior and subsequent measurements (Murage et al. 2007; Stewart et al. 2013). After CO_2 measurements were taken, both sets of samples were flushed with CO_2-free air. The fraction of litter-derived C respired at each CO_2 sampling occasion was estimated using the isotopic mixing model, as follows:

$$f_L = \frac{\delta - \delta_C}{\delta_L - \delta_C} \tag{1}$$

where δ is the $\delta^{13}C$-CO_2 efflux from the litter-amended mineral slurries, δ_C is the average $\delta^{13}C$-CO_2 efflux from the corresponding mineral controls, and δ_L is the average $\delta^{13}C$-CO_2 efflux from the

corresponding litter controls, all at the same time of sampling. Respiration of litter-derived C in CO_2 (CO_{2L}) was calculated by multiplying the f_L value by the total CO_2 efflux:

$$CO_{2L} = f_L \times (CO_2) \tag{2}$$

The remainder of the CO_2 efflux was assigned to respiration of soil-derived C (native C present in the soil prior to incubation).

SOM and plant material measurements

At each harvest, samples were poured over a 250 μm sieve to collect the remaining plant litter, and soil was collected underneath on a 1.2 μm glass microfiber filter under vacuum. Each soil sample was rinsed by passing 100 ml of deionized water through the soil on the filter, with the aim of rinsing away any dissolved organic C or N not associated with the silt or clay. We kept the water used to rinse each sample, and measured its pH using an Orion Expandable ionAnalyzer EA 940 (Thermo-Fisher, Bremen, Germany). Recoveries of silt and clay were all above 94%. Soils were dried at 105 °C and litters were dried at 60 °C. All soil samples were inspected for visible plant fragments that may have passed through the 250 μm sieve, and a representative subset of samples was further inspected using a light microscope, but no visible plant fragments were detected. Oven-dried soils and litters were ground and analysed for $\delta^{13}C$, $\delta^{15}N$, and C and N concentrations on a Costech ECS 4010 (Costech Analytical Technologies, Valencia, CA USA) coupled to a Delta V Advantage IRMS (Thermo-Fisher, Bremen, Germany). The C or N recovered with the soil was defined as MAOM.

Litter-derived C and N in MAOM were calculated according to the same mixing model used for CO_2, substituting the $\delta^{13}C$ and $\delta^{15}N$ values of solid materials (soil fractions or plant litter) from litter-soil slurries and controls. MAOM formation efficiency (FE) was calculated using the following equation:

$$FE = \frac{\text{litter-derived C in MAOM}}{\text{litter-derived C processed}} \tag{3}$$

where litter-derived C in MAOM was defined as that found associated with either the silt or the clay fraction and litter-derived C processed was defined as litter-derived C in MAOM + litter-derived C in CO_2. A

higher value of FE corresponds to more MAOM formed, and less CO_2 produced, per unit of litter processed.

C Saturation calculations

There are multiple regression equations in the literature to estimate the capacity of the silt plus clay fraction of a whole soil to store C. The original equation from Hassink et al. (1997) was calculated using a smaller size cutoff for their silt + clay fractions than we did (0–20 μm compared to our 0–53 μm). Six et al. (2002), performed a similar analysis, but they included separate regressions for studies that used a 0–50 μm size cutoff and showed that the size cutoff had a significant effect on the best fit regression equations. We therefore chose to use a regression equation from Six et al. (2002), rather than from Hassink et al. (1997). We first used their equation for cultivated soils, but calculated negative saturation deficits in some cases, so instead used their equation for grasslands:

$$\text{MAOM-C capacity} = 16.33 + 0.32\,[\text{silt} + \text{clay content}\,(\%)] \tag{4}$$

where silt + clay content is 80.5%, which we measured when we first fractionated the soil. We then calculated saturation deficits according to Stewart et al. (2007):

$$\text{saturation deficit} = 1 - \frac{\text{MAOM-C measured}}{\text{MAOM-C capacity}} \tag{5}$$

where MAOM-C measured is the total C associated with the silt plus clay fractions (C in the two fractions combined) at a given time point, and MAOM-C capacity is from Eq. 4. The saturation deficit is meant to serve as a rough guideline for the saturation level of our soil fractions throughout the incubation, but the results need to be interpreted with caution, as the regressions were calculated using silt plus clay fractions combined, and they may not accurately represent dynamics of the individual silt and clay fractions.

Statistical analysis

We tested for differences in characteristics of roots versus shoots and silt versus clay using two-sample

t-tests. We tested for effects of mineral fraction (i.e., silt vs. clay) and litter type (i.e., shoots vs. roots) on cumulative CO_2-C per g total C separately for each harvest using a two-way ANOVA with Tukey's post hoc test. We tested for effects of mineral fraction (i.e., silt vs. clay) and litter type (i.e., shoots vs. roots) on amounts of litter-derived C and N in MAOM, cumulative litter-derived CO_2-C, per cent litter C respired, per cent litter C processed, and pH, separately for each harvest using two-way ANOVAs with Tukey's post hoc test if the interaction was significant ($p \leq 0.05$). We tested for effects of soil fraction, litter type, and harvest on FE and C/N ratios of litter-derived MAOM using three-way ANOVAs with Tukey's post hoc test if one or more factors were significant ($p \leq 0.05$). Before all t-tests and ANOVAs, we tested for homogeneity of variance using Levene's test. All statistical tests were carried out using the statistical package in R, version 3.3.2 (R Core Team 2016).

Results

Initial litter chemistry and mineral fraction characteristics

Shoots had significantly higher C and N concentrations, but significantly less HWE-N, than roots (Table 1). Shoots also contained more AHF and less AUR, resulting in lower lignocellulose index and AUR/N ratios than roots. However, the amount of HWE-C and the C/N ratios were not significantly different between the two litters.

The SSA of the clay was an order of magnitude higher than that of the silt, both before and after SOM removal by muffling (Table 2). Neither soil fraction showed an appreciable change in SSA with muffling. The clay also had significantly higher Fe_d, Fe_o Al_o, C and N contents than the silt. The estimated C saturation deficit of the initial whole soil was 0.59 (Table 3).

Respiration (CO_2 efflux)

In all samples with added litter, respiration rates were initially high, decreasing rapidly after the first three days. A comparison of cumulative respiration across all litter-soil slurries and controls, normalized to total C at the start of the experiment, is shown in Fig. 1. The

Table 1 Chemical characterization of *Andropogon gerardii* shoots and roots, with corresponding p-values from two-sample t-tests comparing data in each row

	Leaves	Roots	p-value
% C	48.8 ± 0.47	42.5 ± 0.47	< 0.001
% N	1.8 ± 0.02	1.4 ± 0.05	< 0.001
C/N	26.8 ± 0.17	29.6 ± 1.18	0.059
% HWE-C	15.7 ± 0.06	14.4 ± 0.47	0.055
% HWE-N	17.4 ± 0.47	39.9 ± 1.19	< 0.001
C/N of HWE	24.1 ± 0.56	10.6 ± 0.07	< 0.001
% AHF	33.0 ± 0.16	23.6 ± 0.75	< 0.001
% AUR	5.4 ± 0.20	16.4 ± 0.72	< 0.001
AUR/N	3.0 ± 0.11	11.4 ± 0.50	< 0.001
Lignocellulose index	0.14 ± 0.005	0.41 ± 0.02	< 0.001

HWE hot water-extractable, *AHF* acid hydrolyzable fiber, *AUR* acid unhydrolyzable residue. Lignocellulose index is calculated as [acid unhydrolyzable residue/(acid unhydrolyzable residue + acid hydrolyzable fiber)]. Data are means ± one standard error (n = 4 for % C, % N, C/N; n = 3 for % HWE-C, %HWE-N, C/N of HWE; n = 6 for % AHF, % AUR, AUR/N, lignocellulose index)

vast majority of respired C in all slurries with litter was litter-derived, and as a result, slurries with native soil C (litter-soil slurries and mineral controls) had lower cumulative respiration per g of total C than the litter controls throughout the experiment (Fig. 1).

In the short term, there was not a consistent effect of litter type on respiration. Shoot and root litter controls both respired a little over 10% of their total C (Table 4). The shoot-silt slurries respired more CO_2-C than the other litter-soil slurries, none of which were significantly different from one another (Fig. 2a). There was also no clear effect of soil fraction (silt vs. clay) on respiration in the short term. However, slurries with soil respired significantly less than their litter control counterparts, except in the case of shoot-silt slurries (Table 4).

By the end of the incubation (mid term), there was a clear effect of litter type on CO_2-C production from litter-soil slurries, with the shoot-soil slurries respiring significantly less than root-soil slurries (Fig. 2b). The shoot-soil slurries respired a significantly lower percentage of added litter C than all of the other slurries, including litter controls (Table 4). However, there was no significant difference in per cent litter C respired between shoot and root controls.

	Silt	Clay	p-value
SSA, untreated ($m^2 \ g^{-1}$)	12.24 ± 0.14	112.15 ± 1.15	< 0.001
SSA, muffled ($m^2 \ g^{-1}$)	12.17 ± 0.10	114.88 ± 0.94	< 0.001
Fe_d ($mg \ g^{-1}$)	3.21 ± 0.26	14.71 ± 0.70	< 0.001
Fe_o ($mg \ g^{-1}$)	0.21 ± 0.003	0.82 ± 0.003	< 0.001
Al_o ($mg \ g^{-1}$)	0.36 ± 0.01	4.07 ± 0.04	< 0.001
C ($mg \ g^{-1}$)	8.39 ± 0.28	13.13 ± 0.05	< 0.001
N ($mg \ g^{-1}$)	0.83 ± 0.05	1.80 ± 0.00	< 0.001
C/N	10.22 ± 0.31	7.29 ± 0.03	< 0.001
$\delta^{13}C$ (‰)	-18.82 ± 0.46	-18.58 ± 0.04	0.55
$\delta^{15}N$ (‰)	3.27 ± 0.48	9.07 ± 0.09	< 0.001

Table 2 Summary data for soil fractions prior to incubation

SSA specific surface area, *Fe$_d$* dithionite-citrate extractable *Fe*, *Fe$_o$* and *Al$_o$* oxalate-extractable Fe and Al. Data are means ± one standard error (n = 3 for Fe$_d$, Fe$_o$ and Al$_o$; n = 4 for all other measurements)

Table 3 Estimated saturation deficits of soils before the incubation, and in the short term and mid term

	Saturation deficit
Initial	0.59
Short term	
Shoots	0.47
Roots	0.51
Mid term	
Shoots	0.37
Roots	0.47

MAOM formation

Litter-derived MAOM formation occurred in the short term, with between 5.2 and 7.4% of added litter C being recovered in MAOM after 7 days (Table 4). This corresponded to relatively large increases in MAOM-C. In the most extreme case, shoot-silt slurries increased from 0.84% C initially to 1.18% C at day 7, which represents a 40% increase in MAOM-C (Supplemental Table 1). In the short term, more litter-derived MAOM-C was recovered from the shoot-clay slurries, while the other litter-soil slurries all had similar amounts (Fig. 2a). The estimated C saturation deficit of the shoot slurries decreased from the initial 0.59 to 0.47, while that of the root slurries decreased to 0.51 (Table 3). There was a trend of more litter-derived N in MAOM in slurries with roots, but only root-clay slurries were significantly different from shoot-soil slurries (Fig. 3). The C/N ratios of litter-derived MAOM in the short term were lower for slurries with roots than with shoots (Table 5), consistent with there being more root-derived N in MAOM. There was no effect of soil fraction on the C/N of root-derived MAOM, but shoot-clay slurries had a

significantly higher C/N ratio than shoot-silt slurries. The pH of the litter-soil slurries ranged from 7.1 in the root-clay slurries to 8.4 in the shoot-silt slurries (Supplemental Table 1), but pH did not correlate with MAOM formation in the short term.

Litter-derived MAOM formation continued in the mid term, and a significant litter effect emerged, with more shoot-C in MAOM than root-C (Fig. 2b). The estimated C saturation deficit decreased from 0.47 to 0.37 in slurries with shoots, and from 0.51 to 0.47 in slurries with roots (Table 3). There was an effect of soil fraction on MAOM-C in slurries with shoots, but not in slurries with roots (Fig. 2b). Contrary to the short term, there was significantly more shoot-derived N in MAOM than root-derived N, but there was still no effect of soil fraction on litter-derived N in MAOM (Fig. 3). The increase in shoot-derived N stabilization caused the C/N ratios for shoot- and root-derived MAOM to converge by the end of the incubation (Table 5). The one exception was the C/N ratio of the shoot-clay slurries, which did decrease but remained significantly higher than the others. Compared to the short-term, the C/N ratio of the shoot-derived MAOM decreased over time, while the C/N ratio of root-derived MAOM did not change. There were no significant differences in pH of the litter-soil slurry solutions in the mid term; their average pH was 7.6 (Supplemental Table 1).

Total litter processing

In the short term, between 10.4 and 16.6% of added litter C was processed (sum of litter-derived C respired and in MAOM), with litter-soil slurries processing significantly more litter C than litter controls
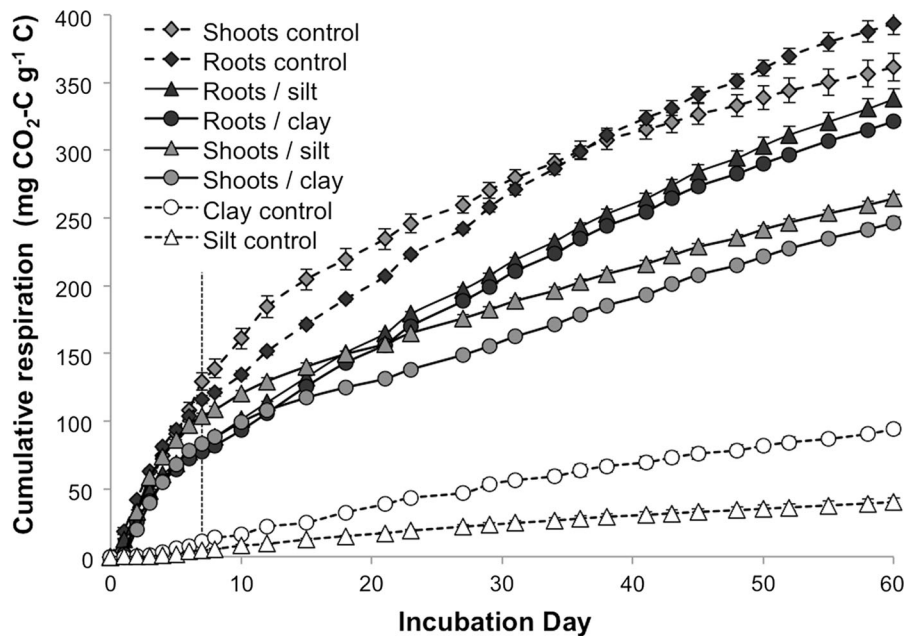
Fig. 1 Cumulative respiration by soil fraction and litter type, including controls, over the 60-day incubation. Values are calculated on a per g C basis to allow direct comparison between samples with and without soil, and with and without litter. Values represent the total amount of CO_2 respired as a proportion of total C present at the start of the experiment.

Litter-soil mixtures started with the most total C, followed by litter controls, and soil controls started with much less total C. Vertical dashed line shows timing of first harvest (day 7; short term). Points are means $\pm$ one standard error (n = 4); some error bars are smaller than the symbols

Table 4 Per cent litter-derived C in respired CO_2 and mineral-associated organic matter (MAOM), and per cent processed (sum of CO_2-C and MAOM-C) in the short term and mid term

Harvest	Litter type	Soil fraction	% litter-derived C respired	% litter-derived C in MAOM	% litter-derived C processed
Short term	Shoots	Silt	10.7 ± 0.2a	5.9 ± 0.1a	16.6 ± 0.3a
		Clay	9.0 ± 0.1bc	7.4 ± 0.2b	16.4 ± 0.2a
		Litter control	10.9 ± 0.6a	–	10.9 ± 0.6b
	Roots	Silt	8.7 ± 0.1bd	5.2 ± 0.4a	14.0 ± 0.5c
		Clay	7.7 ± 0.2	5.3 ± 0.04a	13.0 ± 0.1c
		Litter control	10.4 ± 0.4ac	–	10.4 ± 0.4b
Mid term	Shoots	Silt	31.3 ± 0.3a	13.1 ± 0.3a	44.4 ± 0.6a
		Clay	30.4 ± 0.6a	15.6 ± 0.5b	45.9 ± 1.0ab
		Litter control	36.2 ± 1.0b	–	36.2 ± 1.0c
	Roots	Silt	40.4 ± 1.0c	9.3 ± 0.7c	49.7 ± 1.4b
		Clay	38.6 ± 0.5bc	8.5 ± 0.3c	47.1 ± 0.6ab
		Litter control	39.3 ± 0.9bc	–	39.4 ± 0.9c

Data are means $\pm$ one standard error (n = 4)

Different letters indicate significant differences ($p < 0.05$) between treatments for each variable within each harvest (means were not compared between harvests)

(Table 4). In the litter-soil slurries, more shoot C was processed than root C, but there was no effect of soil fraction on total litter C processing.

At the end of the incubation, between 36.2 and 49.7% of the added litter C was processed, and litter-soil slurries continued to process significantly more
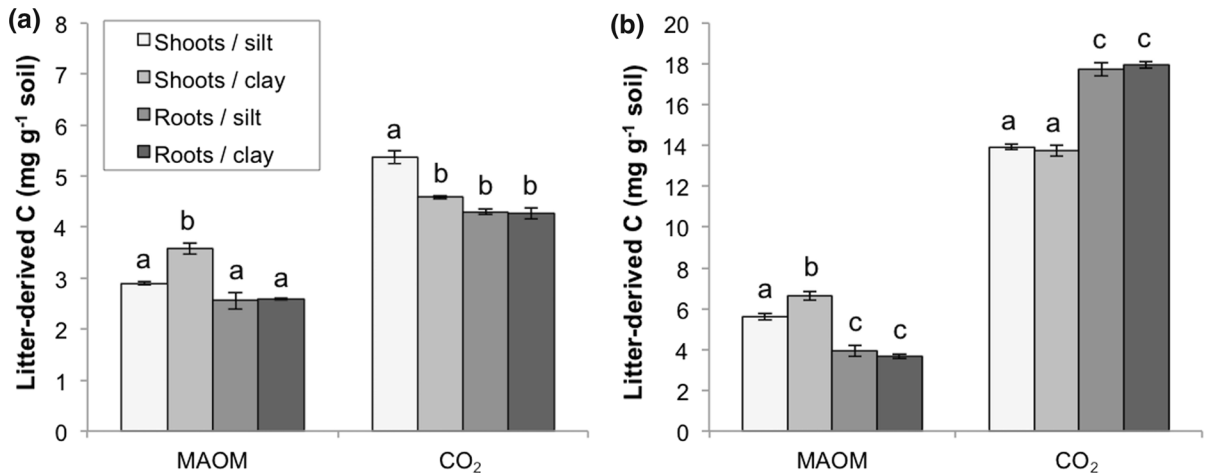
Fig. 2 Litter-derived C in MAOM and CO_2 in litter-soil slurries in the short term (**a**) and mid term (**b**). Data are means ± one standard error (n = 4). Different letters indicate significant differences (p < 0.05) between treatments
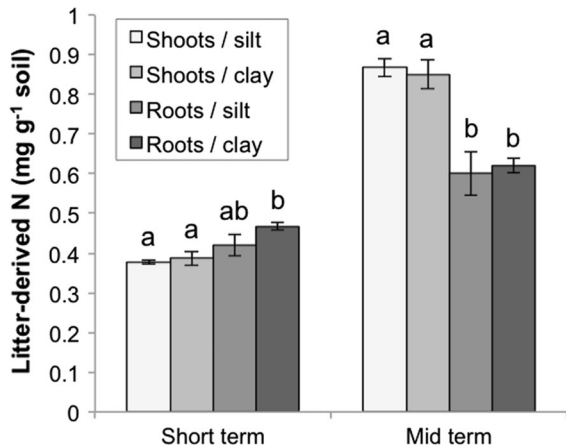

Fig. 3 Litter-derived N in MAOM in litter-soil slurries in the short term and mid term. Data are means ± one standard error (n = 4). Different letters indicate significant differences (p < 0.05) between treatments, within each harvest (means were not compared between harvests)

litter C than litter controls (Table 4). Contrary to the short term, roots were generally more processed than shoots, but the only significant difference was between the shoot-silt and shoot-clay slurries (Table 4). There was still no significant effect of soil fraction on the per cent of litter C processed, similar to the short term.

MAOM formation efficiency

In the short term, the relatively large amount of MAOM-C and low respiration in the shoot-clay slurries led to a significantly higher FE than the other

litter-soil slurries (Table 5). There was not a consistent litter effect, however, as the shoot-silt slurries had the lowest FE in the short term. FEs were generally higher in slurries with clay, but the difference was only significant in slurries with shoots. All of the FEs in the short term, which ranged from 0.36 to 0.45, were significantly higher than in the mid term, when they deceased to between 0.18 and 0.34. There was a clearer litter effect in the mid term than in the short term; shoot-soil slurries had significantly higher FEs than root-soil slurries. However, there was no effect of soil fraction on FEs in the mid term.

Discussion

Effect of litter quality on MAOM formation

Over the 60-day incubation, more MAOM formed from shoots than from roots, as we predicted based on litter chemistry. This occurred despite the fact that the same amount, or more root C was processed than shoot C (Table 4). MAOM formation from shoots was more efficient, with less respired C per g of new MAOM-C, than from roots (Fig. 2b). We predicted that MAOM formation from shoots would be more efficient than from roots in both soil fractions during both phases of decomposition, and this was true for three cases out of four (silt slurries during the short term were the exception). The difference in FE was more pronounced in the mid term, which may be related to the

Table 5 Formation efficiencies (FE) and C/N ratios of litter-derived, mineral-associated organic matter (MAOM)

Harvest	Litter type	Soil fraction	FE	C/N of litter-derived MAOM
Short term	Shoots	Silt	$0.36 \pm 0.005a$	$7.7 \pm 0.1a$
		Clay	$0.45 \pm 0.01b$	$9.3 \pm 0.2b$
	Roots	Silt	$0.38 \pm 0.01ac$	$6.1 \pm 0.4c$
		Clay	$0.41 \pm 0.01c$	$5.6 \pm 0.1c$
Mid term	Shoots	Silt	$0.3 \pm 0.003d$	$6.5 \pm 0.1c$
		Clay	$0.34 \pm 0.005d$	$7.8 \pm 0.3a$
	Roots	Silt	$0.18 \pm 0.01e$	$6.6 \pm 0.3c$
		Clay	$0.18 \pm 0.004e$	$5.9 \pm 0.1c$

Data are means $\pm$ one standard error (n = 4). Different letters indicate significant differences (p < 0.05) between treatments across both harvests

saturation deficit (how far a soil is from its maximum possible C content), or saturation level of the soil fractions through time (Hassink et al. 1997; Six et al. 2002; Stewart et al. 2007). Castellano et al. (2015) proposed that the effect of litter quality on MAOM formation increases as soils move from high to moderate saturation deficits. In the short term, the saturation deficit of these soil fractions was relatively high. We estimated the saturation deficit of our initial soil to be 0.59, but there was also a lack of change in SSA with muffling, which suggests ample surface area available for MAOM formation (Table 2). In the short term, with a high initial soil C saturation deficit, differences in FEs between litter types were not very clear. The addition of new MAOM (increases in MAOM-C of up to 40% from the start of the incubation), lowered the estimated C saturation deficits, at which point clear differences in FEs were observed.

While we observed more shoot- than root-derived MAOM, several previous studies have shown more SOM formation from roots than shoots (Rasse et al. 2005; Jackson et al. 2017). Many of those studies focus on total SOM (e.g., Balesdent and Balabane 1996; Bolinder et al. 1999 and studies reviewed therein), which includes fragmented or partially processed litter such as POM, lumped in with MAOM. Of the few studies that explicitly measured older, more processed SOM, their results generally agree with ours. When Bird et al. (2008) tracked the fate of ^{13}C from decomposing pine needles and fine roots in situ, they observed 28% more total C retained from roots than from needles. But this difference was mainly due

to greater retention of root-C in the POM; in fact, more needle-derived C was retained in what they defined as the humic and humin fractions, which are comparable to the fractions we measured here. In a similar study, Hatton et al. (2015) observed less total C and N retained from needles, but proportionally more from needles than from roots in stable SOM fractions (those with longer ^{14}C mean residence times). Steffens et al. (2015) performed a laboratory incubation using ^{13}C labeled leaves and roots, and did not find differences in MAOM (they use "heavy fraction") after 206 days, when the percentages of added litter C that had been respired were comparable to those in our study. However, they did find a strong correlation between the litter-C recovered in MAOM and water-extractable organic C at some time points, which agrees with our results.

Formation efficiency in the short and mid term

Across all litter-soil treatments, MAOM formation was more efficient in the short term than in the mid term. In the litter-soil slurries, roughly 15% of litter C was processed in the short term (Table 4), which corresponds to the amount of HWE-C in the two litters. This supports previous work that has shown that leaching of soluble plant compounds forms a rapid, efficient microbial pathway to MAOM (Cotrufo et al. 2015; Haddix et al. 2016). It is also possible that some compounds leaching from the litter were stabilized directly by the minerals (see Kramer et al. 2012), which might explain the higher C/N in the shoot treatments during the short term, but we cannot

confirm this with our experimental design. The main input to MAOM during the mid term was likely microbial products derived from decomposition of cellulose (for which AHF can serve as a rough proxy) and other insoluble plant compounds, assuming the soluble compounds had already been transformed or associated directly with the minerals. This shift to decomposition of less readily degradable compounds is reflected in the overall decrease in FE during the later stage. The decrease in FE was larger for roots than for shoots, which corresponds to their higher lignocellulose index.

Comparing soil fractions

We observed some differences in C and N dynamics and MAOM formation between silt and clay, but they were not as consistent across treatments as we expected. In general, the mineralogical properties of the clay were more conducive to MAOM formation than those of the silt, with the clay showing higher SSA and greater amounts of Fe_d, Fe_o and Al_o. The amount of MAOM in a given soil has been found to correlate strongly with poorly crystalline Fe and Al ($Fe_o + Al_o$) (Kleber et al. 2005), or with crystalline Fe ($Fe_d − Fe_o$) (Khomo et al. 2017), or both (Mikutta et al. 2006). In addition, respiration from incubated soils has been found to correlate negatively with Fe_d, Fe_o, and Al_o (Singh et al. 2017a), suggesting a stabilizing effect of Fe and Al oxides and oxyhydroxides on SOM (Kögel-Knabner et al. 2008; Singh et al. 2017b). Given the differences in mineral properties between the soil fractions, we expected clay to have more MAOM formation than silt throughout the incubation. This was true for stabilization of shoot-C, but not for root-C. The two soil fractions accrued similar amounts of root-C, and it was generally less than the amount of shoot-C. This aligns with the ideas in the Castellano et al. (2015) litter quality-saturation framework. The saturation deficits of the slurries with roots were consistently higher than those with shoots, and the saturation deficits may have been high enough that free surface area was not limiting for MAOM formation from roots. In other words, the available surface area for MAOM formation may have greatly exceeded the amount of MAOM formed. Instead, the efficiency of microbial processing may have been the limiting factor for MAOM formation from roots. Interestingly, the two soil fractions did not differ in

their stabilization of litter-derived N. In general, MAOM is enriched in N compared to the plant material from which it is principally derived (Tipping et al. 2016), because N-rich compounds show affinity for mineral surfaces and are especially prone to stabilization (Sollins et al. 2006; Kleber et al. 2007; Knicker 2011). As a result, both soil fractions may have preferentially stabilized available N-rich compounds, resulting in similar amounts of N in each. This result agrees with a meta-analysis from Tipping et al. (Tipping et al. 2016), wherein the authors analysed data from over 2000 samples over a wide range of soils, and found that when the mineral/SOM ratio is high, as in our soil fractions, the C/N ratio is low.

Initial quality of the roots and shoots

Our measured values for % AUR and AUR/N are within the range of those reported for other grasses, reviewed by Rasse et al. (2005). The relatively high HWE-N in the roots may have been due to residual fertilizer remaining on the roots after harvest, which would explain its rapid transformation to MAOM by day 7, and the consistency in the C/N ratio of root-derived MAOM over time. This may also explain why the initial difference in the C/N ratios of the roots and shoots was only marginally significant (p = 0.059). Regardless of their relatively high HWE-N content, the roots used in this study were of lower initial quality than the shoots, based on their % AUR, AUR/N and lignocellulose index (Table 1). Lignocellulose index and AUR/N have been shown to be reliable indicators of litter quality which can be used to predict litter decomposition dynamics (Adair et al. 2008; Soong et al. 2015).

Conclusion

Our data support the Microbial Efficiency-Matrix Stabilization hypothesis of Cotrufo et al. (2013), linking the effects of litter chemistry on microbial efficiency to SOM formation and mineral-association. In this study, more MAOM formed from shoots than from roots, and it is likely because microorganisms processed the shoots more efficiently, respiring less CO_2 and transforming more shoot C and N to MAOM. We did not characterize the microbial communities present, but recent evidence suggests that they can

exert a major control on the formation and stability of SOM (Kallenbach et al. 2016), and the role that microbial community composition plays in determining the efficiency of litter processing and MAOM formation deserves further study. We focused on MAOM only, in the absence of many of those natural processes that might preferentially stabilize root C and N. In fact, root exudates, which were not included in this study, could form a rapid pathway of MAOM formation according to this framework, although they may also lead to priming of MAOM (Keiluweit et al. 2015). This work adds to a growing body of evidence that the recalcitrance of litter does not explain the longevity of litter-derived compounds in soil (Schmidt et al. 2011; Lehmann and Kleber 2015), but that a host of other mechanisms are at play. The proximity of roots to soil surfaces, protection of root material through aggregation, and root exudation might explain why many studies find greater contributions of roots than shoots to total SOM. Despite differences in initial litter chemistry in the shoots and roots, similar amounts of the two litters were processed by the end of the incubation, especially in the litter controls. This suggests that the difference in initial quality did not affect the rate at which the litters were processed, but did affect the efficiency of that processing. "Litter quality" therefore should be defined as an intrinsic property of the litter itself, rather than an extrinsic property determined by the rate of decomposition (Swift et al. 1997). One broad consistency in our experiment that applied irrespective of litter type or soil fraction was the low C/N ratio of the MAOM that formed, which differed greatly from the C/N ratio of the source plant material, and points to the importance of microbial processing as a pathway of MAOM formation.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Abiven S, Recous S, Reyes V, Oliver R (2005) Mineralisation of C and N from root, stem and leaf residues in soil and role of their biochemical quality. Biol Fertil Soils 42:119–128. https://doi.org/10.1007/s00374-005-0006-0

Adair EC, Parton WJ, Del Grosso SJ et al (2008) Simple three-pool model accurately describes patterns of long-term litter decomposition in diverse climates. Glob Chang Biol 14:2636–2660. https://doi.org/10.1111/j.1365-2486.2008.01674.x

Allison SD, Jastrow JD (2006) Activities of extracellular enzymes in physically isolated fractions of restored grassland soils. Soil Biol Biochem 38:3245–3256. https://doi.org/10.1016/j.soilbio.2006.04.011

Anderson DW, Paul EA (1984) Organo-mineral complexes and their study by radiocarbon dating. Soil Sci Soc Am J 48:298–301. https://doi.org/10.2136/sssaj1984.03615995004800020014x

Balesdent J, Balabane M (1996) Major contribution of roots to soil carbon storage inferred from maize cultivated soils. Soil Biol Biochem 28:1261–1263. https://doi.org/10.1016/0038-0717(96)00112-5

Balesdent J, Mariotti A, Guillet B (1987) Natural C-13 abundance as a tracer for studies of soil organic-matter dynamics. Soil Biol Biochem 19:25–30. https://doi.org/10.1016/0038-0717(87)90120-9

Balesdent J, Balesdent J, Mariotti A et al (1996) Measurement of soil organic matter turnover using ^{13}C natural abundance. In: Boutton TW, Yamasaki S-I (eds) Mass spectrometry of soils. Marcel Dekker Inc, New York, pp 83–111

Batjes NH (2014) Total carbon and nitrogen in the soils of the world. Eur J Soil Sci 65:4–21. https://doi.org/10.1111/ejss.12114_2

Birch HF (1958) The effect of soil drying on humus decomposition and nitrogen availability. Plant Soil 10:9–31. https://doi.org/10.1007/BF01343734

Bird JA, Kleber M, Torn MS (2008) ^{13}C and ^{15}N stabilization dynamics in soil organic matter fractions during needle and fine root decomposition. Org Geochem 39:465–477. https://doi.org/10.1016/j.orggeochem.2007.12.003

Birge HE, Conant RT, Follett RF et al (2015) Soil respiration is not limited by reductions in microbial biomass during long-term soil incubations. Soil Biol Biochem 81:304–310. https://doi.org/10.1016/j.soilbio.2014.11.028

Bolinder MA, Angers DA, Giroux M, Laverdière MR (1999) Estimating C inputs retained as soil organic matter from corn (*Zea mays* L.). Plant Soil 215:85–91. https://doi.org/10.1023/A:1004765024519

Calderón FJ, Reeves JB III, Collins HP, Paul EA (2011) Chemical differences in soil organic matter fractions determined by diffuse-reflectance mid-infrared spectroscopy. Soil Sci Soc Am J 75:568–579. https://doi.org/10.2136/sssaj2009.0375

Cambardella CA, Elliott ET (1992) Particulate soil organic-matter changes across a grassland cultivation sequence. Soil Sci Soc Am J 56:777–783. https://doi.org/10.2136/sssaj1992.03615995005600030017x

Castellano MJ, Mueller KE, Olk DC et al (2015) Integrating plant litter quality, soil organic matter stabilization, and the

carbon saturation concept. Glob Chang Biol 21:3200–3209. https://doi.org/10.1111/gcb.12982

Christensen BT (2001) Physical fractionation of soil and structural and functional complexity in organic matter turnover. Eur J Soil Sci 52:345–353. https://doi.org/10.1046/j.1365-2389.2001.00417.x

Cotrufo MF, Wallenstein MD, Boot CM et al (2013) The microbial efficiency-matrix stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: do labile plant inputs form stable soil organic matter? Glob Chang Biol 19:988–995. https://doi.org/10.1111/gcb.12113

Cotrufo MF, Soong JL, Horton AJ et al (2015) Formation of soil organic matter via biochemical and physical pathways of litter mass loss. Nat Geosci 8:776–779. https://doi.org/10.1038/ngeo2520

Crow SE, Swanston CW, Lajtha K et al (2007) Density fractionation of forest soils: methodological questions and interpretation of incubation results and turnover time in an ecosystem context. Biogeochemistry 85:69–90. https://doi.org/10.1007/s10533-007-9100-8

Feng X, Simpson AJ, Simpson MJ (2005) Chemical and mineralogical controls on humic acid sorption to clay mineral surfaces. Org Geochem 36:1553–1566. https://doi.org/10.1016/j.orggeochem.2005.06.008

Fisher BA (2016) Geomorphic controls on mineral weathering, elemental transport, carbon cycling, and production of mineral surface area in a schist bedrock weathering profile, Piedmont Pennsylvania. Doctoral dissertation. University of Minnesota. Minneapolis

Goering HK, Van Soest PJ (1970) Forage fiber analyses (apparatus, reagents, prcedures, and some applications). In: USDA agriculture handbook no. 379. Washington, D.C

Grandy AS, Neff JC (2008) Molecular C dynamics downstream: the biochemical decomposition sequence and its impact on soil organic matter structure and function. Sci Total Environ 404:297–307. https://doi.org/10.1016/j.scitotenv.2007.11.013

Haddix ML, Plante AF, Conant RT et al (2011) The role of soil characteristics on temperature sensitivity of soil organic matter. Soil Sci Soc Am J 75:56–68. https://doi.org/10.2136/sssaj2010.0118

Haddix ML, Paul EA, Cotrufo MF (2016) Dual, differential isotope labeling shows the preferential movement of labile plant constituents into mineral-bonded soil organic matter. Glob Chang Biol 22:2301–2312. https://doi.org/10.1111/gcb.13237

Hassink J, Whitmore AP, Kubat J (1997) Size and density fractionation of soil organic matter and the physical capacity of soils to protect organic matter. Eur J Agron 7:189–199. https://doi.org/10.1016/S1161-0301(97)00045-2

Hatton P-J, Castanha C, Torn MS, Bird JA (2015) Litter type control on soil C and N stabilization dynamics in a temperate forest. Glob Chang Biol 21:1358–1367. https://doi.org/10.1111/gcb.12786

Jackson RB, Lajtha K, Crow SE et al (2017) The ecology of soil carbon: pools, vulnerabilities, and biotic and abiotic controls. Annu Rev Ecol Evol Syst. https://doi.org/10.1146/annurev-ecolsys-112414-054234

Jagadamma S, Steinweg JM, Mayes MA et al (2013) Decomposition of added and native organic carbon from physically separated fractions of diverse soils. Biol Fertil Soils 50:613–621. https://doi.org/10.1007/s00374-013-0879-2

Kallenbach CM, Frey SD, Grandy AS (2016) Direct evidence for microbial-derived soil organic matter formation and its ecophysiological controls. Nat Commun 7:13630. https://doi.org/10.1038/ncomms13630

Keiluweit M, Bougoure JJ, Nico PS et al (2015) Mineral protection of soil carbon counteracted by root exudates. Nat Clim Chang 5:588–595. https://doi.org/10.1038/nclimate2580

Khomo L, Trumbore S, Bern CR, Chadwick OA (2017) Time-scales of C turnover in soils with mixed crystalline mineralogies, Kruger National Park, South Africa. SOIL 3:17–30. https://doi.org/10.5194/soil-3-17-2017

Kleber M, Mikutta R, Torn MS, Jahn R (2005) Poorly crystalline mineral phases protect organic matter in acid subsoil horizons. Eur J Soil Sci 56:717–725. https://doi.org/10.1111/j.1365-2389.2005.00706.x

Kleber M, Sollins P, Sutton R (2007) A conceptual model of organo-mineral interactions in soils: self-assembly of organic molecular fragments into zonal structures on mineral surfaces. Biogeochemistry 85:9–24. https://doi.org/10.1007/s10533-007-9103-5

Klotzbücher T, Kaiser K, Guggenberger G et al (2011) A new conceptual model for the fate of lignin in decomposing plant litter. Ecology 92:1052–1062. https://doi.org/10.1890/10-1307.1

Knicker H (2011) Soil organic N—an under-rated player for C sequestration in soils? Soil Biol Biochem 43:1118–1129. https://doi.org/10.1016/j.soilbio.2011.02.020

Kögel-Knabner I, Guggenberger G, Kleber M et al (2008) Organo-mineral associations in temperate soils: integrating biology, mineralogy, and organic matter chemistry. J Plant Nutr Soil Sci 171:61–82. https://doi.org/10.1002/jpln.200700048

Kramer MG, Sanderman J, Chadwick OA et al (2012) Long-term carbon storage through retention of dissolved aromatic acids by reactive particles in soil. Glob Chang Biol 18:2594–2605. https://doi.org/10.1111/j.1365-2486.2012.02681.x

Lehmann J, Kleber M (2015) The contentious nature of soil organic matter. Nature 528:60–68. https://doi.org/10.1038/nature16069

Leifeld J (2006) Soils as sources and sinks of greenhouse gases. Geol Soc Lond Spec Publ 266:23–44. https://doi.org/10.1144/GSL.SP.2006.266.01.03

Manzoni S, Taylor P, Richter A et al (2012) Environmental and stoichiometric controls on microbial carbon-use efficiency in soils. New Phytol 196:79–91. https://doi.org/10.1111/j.1469-8137.2012.04225.x

Martin RE, Asner GP, Ansley RJ, Mosier AR (2003) Effects of woody vegetation encroachment on soil nitrogen oxide emissions in a temperate savanna. Ecol Appl 13:897–910. https://doi.org/10.1890/1051-0761(2003)13[897:EOWVEO]2.0.CO;2

McKeague JA, Day DH (1966) Dithionite- and oxalate-extractable Fe and Al as aids in differentiating various classes of soils. Can J Soil Sci 46:13–22. https://doi.org/10.4141/cjss66-003

McKee GA, Soong JL, Calderón F et al (2016) An integrated spectroscopic and wet chemical approach to investigate grass litter decomposition chemistry. Biogeochemistry 128:107–123. https://doi.org/10.1007/s10533-016-0197-5

Mehra OP, Jackson ML (1960) Iron oxide removal from soils and clays by a dithionite–citrate system buffered with sodium bicarbonate. Clays Clay Miner 7:317–327. https://doi.org/10.1346/CCMN.1958.0070122

Mikutta R, Kleber M, Torn MS, Jahn R (2006) Stabilization of soil organic matter: association with minerals or chemical recalcitrance? Biogeochemistry 77:25–56. https://doi.org/10.1007/s10533-005-0712-6

Murage EW, Voroney P, Beyaert RP (2007) Turnover of carbon in the free light fraction with and without charcoal as determined using the ^{13}C natural abundance method. Geoderma 138:133–143. https://doi.org/10.1016/j.geoderma.2006.11.002

Paul EA (2016) The nature and dynamics of soil organic matter: plant inputs, microbial transformations, and organic matter stabilization. Soil Biol Biochem 98:109–126. https://doi.org/10.1016/j.soilbio.2016.04.001

Plante AF, Conant RT, Stewart CE et al (2006) Impact of soil texture on the distribution of soil organic matter in physical and chemical fractions. Soil Sci Soc Am J 70:287–296. https://doi.org/10.2136/sssaj2004.0363

Preston CM, Trofymow JA (2015) The chemistry of some foliar litters and their sequential proximate analysis fractions. Biogeochemistry 126:197–209. https://doi.org/10.1007/s10533-015-0152-x

R Core Team (2016). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. https://www.R-project.org/

Rasse DP, Rumpel C, Dignac M-F (2005) Is soil carbon mostly root carbon? Mechanisms for a specific stabilisation. Plant Soil 269:341–356. https://doi.org/10.1007/s11104-004-0907-y

Sanderman J, Maddern T, Baldock J (2014) Similar composition but differential stability of mineral retained organic matter across four classes of clay minerals. Biogeochemistry 121:409–424. https://doi.org/10.1007/s10533-014-0009-8

Schmidt MWI, Torn MS, Abiven S et al (2011) Persistence of soil organic matter as an ecosystem property. Nature 478:49–56. https://doi.org/10.1038/nature10386

Singh M, Sarkar B, Biswas B et al (2017) Relationship between soil clay mineralogy and carbon protection capacity as influenced by temperature and moisture. Soil Biol Biochem 109:95–106. https://doi.org/10.1016/j.soilbio.2017.02.003

Sinsabaugh RL, Manzoni S, Moorhead DL, Richter A (2013) Carbon use efficiency of microbial communities: stoichiometry, methodology and modelling. Ecol Lett 16:930–939. https://doi.org/10.1111/ele.12113

Six J, Elliott ET, Paustian K (1999) Aggregate and soil organic matter dynamics under conventional and no-tillage systems. Soil Sci Soc Am J 63:1350–1358. https://doi.org/10.2136/sssaj1999.6351350x

Six J, Conant RT, Paul EA, Paustian K (2002) Stabilization mechanisms of soil organic matter: implications for C-saturation of soils. Plant Soil 241:155–176. https://doi.org/10.1023/A:1016125726789

Smith P, Cotrufo MF, Rumpel C et al (2015) Biogeochemical cycles and biodiversity as key drivers of ecosystem services provided by soils. SOIL 2:665–685. https://doi.org/10.5194/soild-2-537-2015

Sollins P, Swanston C, Kleber M et al (2006) Organic C and N stabilization in a forest soil: evidence from sequential density fractionation. Soil Biol Biochem 38:3313–3324. https://doi.org/10.1016/j.soilbio.2006.04.014

Soong JL, Reuss D, Pinney C et al (2014) Design and operation of a continuous ^{13}C and ^{15}N labeling chamber for uniform or differential, metabolic and structural, plant isotope labeling. J Vis Exp 83:e51117. https://doi.org/10.3791/51117

Soong JL, Parton WJ, Calderón F et al (2015) A new conceptual model on the fate and controls of fresh and pyrolyzed plant litter decomposition. Biogeochemistry 124:27–44. https://doi.org/10.1007/s10533-015-0079-2

Steffens C, Helfrich M, Joergensen RG et al (2015) Translocation of ^{13}C-labeled leaf or root litter carbon of beech (*Fagus sylvatica* L.) and ash (*Fraxinus excelsior* L.) during decomposition—a laboratory incubation experiment. Soil Biol Biochem 83:125–137. https://doi.org/10.1016/j.soilbio.2015.01.015

Stewart CE, Paustian K, Conant RT et al (2007) Soil carbon saturation: concept, evidence and evaluation. Biogeochemistry 86:19–31. https://doi.org/10.1007/s10533-007-9140-0

Stewart CE, Plante AF, Paustian K et al (2008) Soil carbon saturation: linking concept and measurable carbon pools. Soil Sci Soc Am J 72:379–392. https://doi.org/10.2136/sssaj2007.0104

Stewart CE, Zheng J, Botte J, Cotrufo MF (2013) Co-generated fast pyrolysis biochar mitigates green-house gas emissions and increases carbon sequestration in temperate soils. GCB Bioenergy 5:153–164. https://doi.org/10.1111/gcbb.12001

Swift MJ, Heal OW, Anderson JM (1997) Decomposition in terrestrial ecosystems. University of California Press, Berkeley

Tipping E, Somerville CJ, Luster J (2016) The C:N:P: S stoichiometry of soil organic matter. Biogeochemistry 130:117–131. https://doi.org/10.1007/s10533-016-0247-z

Tisdall J, Oades J (1982) Organic matter and water-stable aggregates in soils. J Soil Sci 33:141–163. https://doi.org/10.1111/j.1365-2389.1982.tb01755.x

Torn MS, Trumbore SE, Chadwick OA et al (1997) Mineral control of soil organic carbon storage and turnover. Nature 389:170–173. https://doi.org/10.1038/38260

Voroney RP, Paul EA, Anderson DW (1989) Decomposition of wheat straw and stabilization of microbial products. Can J Soil Sci 69:63–77. https://doi.org/10.4141/cjss89-007

Wallenstein MD, Haddix ML, Lee DD et al (2012) A litter-slurry technique elucidates the key role of enzyme production and microbial dynamics in temperature sensitivity of organic matter decomposition. Soil Biol Biochem 47:18–26. https://doi.org/10.1016/j.soilbio.2011.12.009

Wattel-Koekkoek E, Van GP, Buurman P, van Lagen B (2001) Amount and composition of clay-associated soil organic matter in a range of kaolinitic and smectitic soils. Geoderma 99:27–49. https://doi.org/10.1016/S0016-7061(00)00062-8