# Plant litter quality affects the accumulation rate, composition, and stability of mineral-associated soil organic matter

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ABSTRACT

Mineral-associated organic matter (MAOM) is a relatively large and stable fraction of soil organic matter (SOM). Plant litters with high rates of mineralization (high quality litters) are hypothesized to promote the accumulation of MAOM with greater efficiency than plant litters with low rates of mineralization (low-quality litters) because litters with high rates of mineralization maximize the synthesis of microbial products and most MAOM is microbial-derived. However, the effect of litter quality on MAOM is inconsistent. We conducted four repeated short-term incubations (46-d each) of four plant litters (alfalfa, oats, maize and soybean) in two low-carbon subsoils (sandy loam and silty loam) with and without nutrient addition. Our short-term incubations focused on the initial stage of litter decomposition during the time when litter quality has a measureable effect on mineralization rates. Plant litter quality had a much greater effect on litter-C mineralization rate and MAOM-C accumulation than did soil type or nutrient addition. Soils amended with high-quality oat and alfalfa litters had greater MAOM-C accumulation than soils amended with low-quality maize and soybean litters. However, soils amended with high-quality litters also had greater litter-C mineralization than soils amended with low-quality litters. As a result, the accumulation of MAOM-C per unit of litter-C mineralization was lower in soils amended with high-vs. low-quality litters (0.65 vs. 1.39 g MAOM-C accumulated $g^{-1}$ C mineralized). Cellulose and hemicelluose indices of accumulated MAOM were greater for maize and soybean than oats and alfalfa, however, most carbohydrates in MAOM were plant-derived regardless of litter quality. At the end of the incubations, more of the accumulated MAOM-N was potentially mineralizable in soils amended with high quality litters. Nevertheless, most of the litter-C remained as residual litter; just 12% was mineralized to $CO_2$ and 13% was transferred to MAOM. Our results demonstrate several unexpected effects of litter quality on MAOM stabilization including the direct stabilization of plant-derived carbohydrates.

## 1. Introduction

The accumulation and mineralization of SOM in the mineral soil matrix (i.e., mineral-associated organic matter; MAOM) is critical to ecosystem function. Due to chemical association with fine mineral soil particles, MAOM is relatively stable compared to bulk SOM (Marschner et al., 2008; von Lutzow et al., 2007). However, MAOM is also an important source of nutrients to plants and microbes (Cates and Ruark, 2017; Kallenbach et al., 2015). Mineral-associated organic matter can serve both functions because it typically accounts for more than 50% of total SOM (Beare et al., 2014; Stewart et al., 2008).

Plant litter quality can affect the stabilization and mineralization of MAOM, ultimately impacting soil quality and crop production (Cyle et al., 2016; Drinkwater et al., 1998; Kallenbach et al., 2015; Kirchmann et al., 2004). Recent concepts suggest high-quality plant litters characterized by rapid decomposition rates, low C/N ratios, and low phenol concentrations should lead to faster and more efficient accumulation of MAOM than low-quality plant litters characterized by slow decomposition rates, high C/N ratios, and high phenol concentrations (Cotrufo et al., 2013). However, a review of well-controlled experiments determined that the accumulation of high-quality litter-C in the mineral soil matrix is not consistently faster nor more efficient than the accumulation of low-quality plant litter-C (Castellano et al., 2015).

The lack of a consistent effect of plant litter quality on the rate and efficiency of MAOM accumulation is surprising because the concept has a strong foundation in ecological theory. Metabolic theory of ecology predicts high-quality plant litters should promote microbial C use efficiency, biomass, and growth rate (Brown et al., 2004; Xu et al., 2013); and a broad array of data suggests most MAOM is comprised of microbial rather than plant residues (Kögel-Knabner, 2002; Miltner et al., 2012; Sollins et al., 2009). Thus, given equal inputs of high and low quality litters, high quality litters should produce more MAOM because they yield more microbial products of metabolism and these compounds are thought to comprise the majority of MAOM (Bradford et al., 2013; Kallenbach et al., 2015; Manzoni et al., 2012; Schimel and Schaeffer, 2012).

At present, the inconsistent effects of plant litter quality on the rate and efficiency of MAOM accumulation have been partially attributed to methodological limitations, including confounding experimental factors that interact with litter quality as well as the ability to accurately assign MAOM to microbial vs. plant origin (Castellano et al., 2015). Field comparisons of live plants unavoidably confound the effects of litter quality with the effects of litter amount and plant phenology (i.e., when litter is deposited into the soil). No two species deposit equal amounts of litter at identical rates. Different amounts and timings of litter input alter environmental conditions such as temperature and moisture, which have large and complex direct and indirect effects on microbial physiology (Manzoni et al., 2012; Schimel and Schaeffer, 2012). These effects complicate *in situ* isolation of litter quality effects on the rate and efficiency of MAOM accumulation.

In addition to potential complications with field experiments, modern analytical techniques to estimate the composition and source of MAOM may underestimate the contribution of plant-derived compounds including cutin, suberin, and lignin-derived phenols due to poor extraction efficiency. For example, Hernes et al. (2013) estimated that the CuO technique extracts less than 50% of MAOM phenols. Lin and Simpson (2016) estimated that 81–98% of cutin and suberin biomarkers were not extractable with KOH/MeOH hydrolysis. The extraction efficiencies following these oxidation and hydrolysis reactions are also sensitive to a variety of factors that vary across laboratories including reaction temperatures and pressures, the concentration and presence of different reactants, and the method of extraction (Angst et al., 2017a,b; Goni and Montgomery, 2000; Kaiser and Benner, 2012).

In addition, growing evidence and theory support an important role for the direct stabilization of plant residues in MAOM, particularly in nutrient poor soils and later phases of litter decomposition (Cotrufo et al., 2015; Liang et al., 2017). In nutrient-poor soils, MAOM often contains substantial amounts of plant biomolecules (Angst et al., 2017a,b; Gillespie et al., 2014; Sanderman et al., 2014). In later phases of decomposition, direct stabilization of depolymerized structural plant residues can drive MAOM accumulation (Cotrufo et al., 2015). Indeed, the presence of cellulosic materials in MAOM has been confirmed through strong acid extractions designed to hydrolyze cellulose, as reviewed by Chantigny and Angers (2007). More specifically, Puget et al. (1999) used a strong-acid extraction of MAOM after prior removal of particulate organic matter to identify a glucose-dominated suite of carbohydrate monomers (suggesting cellulosic sources); by contrast, a weak-acid extraction targeting noncellulosic materials extracted smaller amounts of glucose and more balanced distributions of other carbohydrate monomers from the same MAOM. Although this work could not evaluate whether cellulosic material was physically protected or instead chemically bound within MAOM, it is likely that microbial activity will partially depolymerize structural polysaccharides such as cellulose into more soluble fragments, which would facilitate their incorporation into MAOM.

In both nutrient-poor soils and later phases of decomposition, plant litter quality may have unexpected effects on MAOM. For example, if the concentration of lignin in plant litter is proportional to the release of lignin monomers during depolymerization and their subsequent retention in MAOM, then low-quality (i.e., high lignin) plant litters may lead to greater accumulation of plant-derived MAOM, potentially counterbalancing the lower accumulation of microbial-derived MAOM (as compared to high-quality litters). Alternatively, if depolymerization and retention of cutin, suberin, or lignin is higher in the presence of nutrients and labile substrates (Klotzbücher et al., 2011; Talbot et al., 2012), then high-quality plant litters may lead to greater accumulation of both plant-derived and microbial-derived MAOM. Thus, to understand how plant litter quality impacts MAOM accumulation, research must clarify the factors that regulate retention of plant-derived biomolecules in MAOM.

Ultimately, three main factors interact to determine the amount and stability of SOM: abiotic environment (microclimate and soil type), biological activity, and type of organic matter input (Kögel-Knabner, 2014). Given the potential for these factors to interact, our objective was to isolate the effect of organic matter input quality on the rate and efficiency of MAOM accumulation during the initial phase of decomposition when the rate of litter decomposition is most different across litter types. We incubated four plant litters in two soil types with and without the addition of a nutrient solution. We hypothesized that: i) high-quality plant litters promote more efficient accumulation of MAOM-C and -N than do low-quality plant litters; ii) nutrient addition, by similarly enhancing microbial C use efficiency, increases the proportion of litter that is transferred to MAOM; iii) MAOM in soils incubated with high-quality litters has a greater proportion of microbial-derived carbohydrates than does MAOM in soils incubated with low quality litters, and iv) because MAOM is stabilized due to physicochemical properties of minerals, neither plant litter quality nor nutrient addition affects the potential mineralization (i.e., stability) of litter-N accumulated in MAOM-N.

## 2. Materials and methodology

### 2.1. Soil sampling and preparation

Subsoils of two distinct soil series were sampled: a Clarion fine loam (mixed superactive, mesic Typic Hapludoll) located in Story County, Iowa (42°6′ N, 93°35′W) and a Fayette fine silt (mixed, superactive, mesic Typic Hapludalf) located in Fayette County, Iowa (42°56′ N, 91°46′W). The clay fraction of both soils is generally composed of 2:1 clay minerals from the smectite group. Land use for both soils was unfertilized perennial lawn turf grass (a mixture of C3 species) for > 10 years. At each location, subsoils were sampled from 50 to 100 cm depth and homogenized. This depth corresponded to a B horizon material. From here forward, the Clarion and Fayette subsoils are referred to as 'sandy loam' and 'silty loam' respectively. The properties of the sandy loam soil were: pH, 7.1 (1:1$H_2O$); sand, 710 g $kg^{-1}$; clay, 160 g $kg^{-1}$; silt, 140 g $kg^{-1}$. The properties of the silty loam were: pH, 5.1 (1:1$H_2O$); sand, 320 g $kg^{-1}$; clay, 320 g $kg^{-1}$; silt, 370 g $kg^{-1}$. Although soil mineralogy is well known to affect soil organic matter accumulation, the purpose of selecting two soil types in this study was to determine whether the effects of plant litter were consistent across more than one soil type. After sampling, the subsoils were air-dried to constant mass and passed through a 2-mm sieve. Two subsamples from each soil were taken, one for particle size analysis and one for chemical analyses (Table 1). These samples are henceforth referred to as 'whole soil.' The soils at the sampled depths did not contain measurable carbonates.

### 2.2. Plant sampling and preparation

Four plant litters comprised of leaves and stems were collected from the Iowa State University Agriculture Research Farm in Boone County, Iowa (42° 00 'N; 93°46' W). The litters included: alfalfa (*Medicago sativa* L.), maize (*Zea mays* L.), oats (*Avena sativa* L.), and soybean (*Glycine max* L. Merr). Plant litters were sampled to represent their biochemical

**Table 1**
Soil and plant properties prior the incubation.

| | $\delta^{13}C$ (VPDB) | Organic C | Total N | Total Carbohydrates | Total Lignin | C/N ratio | (galactose + mannose)/(arabinose + xylose)[a] |
|---|---|---|---|---|---|---|---|
| **Whole soil** | | g $kg^{-1}$ whole soil | | | | | |
| Sandy loam | −13.46 | 2.48 | 0.22 | 1.56 | 0.29 | 11.46 | 0.36 |
| Silty loam | −19.49 | 2.58 | 0.40 | 1.81 | 0.80 | 6.46 | 0.43 |
| **MAOM**[b] | | g $kg^{-1}$ soil < 53 μm | | | | | |
| Sandy loam | −17.73 | 4.06 | 0.47 | 1.21 | 0.15 | 8.72 | 0.92 |
| Silty loam | −20.6 | 2.87 | 0.39 | 0.88 | 0.48 | 7.29 | 1.05 |
| **Plant litter** | | g $kg^{-1}$ litter | | | | | |
| Maize | −11.81 | 437 | 5.6 | 197 | 38.2 | 77.9 | 0.06 |
| Soybean | −28.08 | 452 | 5.09 | 156 | 31.2 | 88.7 | 0.23 |
| Oats | −30.68 | 387 | 17.3 | 172 | 12.6 | 22.4 | 0.09 |
| Alfalfa | −30.38 | 418 | 25.3 | 131 | 15.8 | 16.5 | 0.38 |

[a] Higher ratios indicate greater proportions of microbial-derived carbohydrates.
[b] Mineral-associated organic matter.

composition when normally incorporated into soil and to maximize differences in litter quality, which varies throughout growth. Maize and soybean were sampled post-senescence prior to harvest, while oats was sampled at the time it would be terminated if grown as a cover crop (before seed production and still green), and alfalfa was sampled after the third cutting in the second year of growth (when it would typically be plowed into the soil). Plant litter samples were oven dried at 60 °C for 48 h then ground to pass a 1-mm sieve.

### 2.3. Incubation

We conducted a fully factorial experiment consisting of two soils (sandy loam and silty loam), four plant litters (maize, soybean, oats, and alfalfa), and two inorganic nutrient additions (with and without nutrient addition; see below), replicated four times. There was a total of 80 samples including four replicate no-litter controls for each soil x nutrient combination.

We followed the sieving/winnowing experimental procedure established by Kirkby et al. (2011, 2013, 2014). Samples were incubated for four, 46-day (d) cycles. The length of the cycle (46 d) was chosen based on a pilot study that determined the time required for $CO_2$ production to reach an asymptote (Fig. S1). At the beginning of each cycle, plant litter was added to the air-dried soil at a rate of 8.7 g C $kg^{-1}$ dry soil and homogenized to minimize formation of microsites with high C concentration. The amount of plant litter-C added was calculated based on previous experiments (Martens, 2000). At the end of each 46-d incubation cycle, the soil was air-dried and the remaining litter was removed with winnowing which consists of using a gentle stream of air to remove low-density litters (Kirkby et al., 2013). This method allowed us to focus on the short-term effect of litter quality on the accumulation of mineral-associated organic matter (MAOM). Although this experimental design does not simulate natural conditions, it provides an opportunity to maximize microbial activity, accurately measure the quantity and chemistry of plant litter transferred to stable SOM, and isolate the effects of plant litter quality, soil type and nutrient addition (Kirkby et al., 2013, 2014; Zhu et al., 2017).

Each sample (soil x plant litter x nutrient solution) was incubated in a 9.6 cm diameter x 7 cm high Buchner funnel. On the bottom of the funnel we placed a glass fiber filter paper (Whatman GD, 90 mm in diameter) followed by glass wool and another glass filter paper. On top of these filters, we placed the soil mixture (Lewis et al., 2014). Each sample contained 100 g of dry soil, 0.869 g of plant litter-C and 50 g of non-native coarse sand. The coarse sand was acid washed with 1 M HCl and subsequently 0.01 M $CaCl_2$ prior to the incubation, and was added to maintain aeration. The soils were incubated at 70% water holding capacity based on a pre-incubation study using both subsoil types without plant litter addition to determine their respective water holding capacity that maximized C mineralization. As a result, the volume of water added to each subsoil differed. Although incubation at a common water potential would be ideal, we used this method (Robertson et al., 1999) because it serves as a relatively accurate proxy for water potential (Robertson et al., 1999; Castellano et al., 2011) and our research focus was litter quality rather than soil type. Thus, comparisons of absolute numbers across soil type (rather than relative differences) should be interpreted with caution.

After initial soil wetting at the beginning of each of the four incubation cycles, half of the soil samples received a nutrient solution containing essential nutrients (1.0 mM $K_2SO_4$, 2.0 mM $KH_2PO_4$, 1.0 mM $MgSO_4$, 2.0 μM $MnSO_4$, 2.0 μM $ZnSO_4$, 0.5 μM $CuSO_4$, 14 mM $NH_4NO_3$, 4.0 mM $CaCl_2$, 25 μM $H_3BO_3$, 0.5 μM $Na_2MoO_4$). The solution was designed to alleviate nutrient limitation of microbes during long-term incubations (Nadelhoffer, 1990).

Samples were incubated in the dark at 24 °C and 50–60% relative humidity. Water holding capacity was maintained throughout each incubation cycle by monitoring the change in mass of each sample and adjusting the mass loss by the addition of deionized water or nutrient solution every other day.

During each of the four 46-d incubations, soil $CO_2$-C production was measured with an infrared gas analyzer (LiCOR, Lincoln, NE, USA). Measurements were daily in the first eight days (0–7 d), then every three days (10, 13, 16 d), and finally every ten days (26, 36, 46 d) for all four incubation cycles. Before each measurement, the incubating soil was placed in a closed container, and the increase in $CO_2$-C concentration in the headspace of the container was converted into a efflux, as the instantanous respiration (i.e., mg $CO_2$-C kg soil $day^{-1}$). The duration of container closure depended on the expected efflux. Cumulative $CO_2$-C emissions from each 46-d incubation cycle were calculated from individual efflux measurements using linear interpolation and summed across all four 46-d cycles (184-d), and converted into a cumulative efflux. To account for plant litter vs. native soil C mineralization, we subtracted $CO_2$ effluxes from no-litter controls for each corresponding soil type x nutrient treatment. This allowed us to account for the decomposition of native soil C, especially in low-nutrient soils (Fontaine et al., 2004; Kirkby et al., 2013). Plant litter C not mineralized to $CO_2$ or transferred to MAOM was removed by winnowing and considered as particulate organic matter.

### 2.4. Soil and plant analyses

A 15-g subsample was taken from each soil sample prior to the incubation study and at the end of the fourth incubation cycle (i.e., initial and final conditions). These soil subsamples were air-dried and residual litter was removed by winnowing. The subsamples were then passed through a 2-mm sieve and mixed with sodium hexametaphosphate

solution at a ratio of 3 (45 ml of solution): 1 (15 g of soil), and shaken for 2 h in a reciprocating shaker at 120 reciprocations per minute. The shaken solution was then passed through a 53 μm sieve to separate the < 53 μm fraction, henceforth referred to as the mineral associated organic matter soil fraction (i.e., MAOM). The MAOM and > 53 μm soil fractions were oven-dried at 60 °C for 48 h. The dried MAOM fraction was finely ground and used for chemical analyses. For a subset of samples, the > 53 μm soil fraction was further fractionated by density using sodium polytungstate with a density of 2.0 g $cm^{-3}$. We then recovered the 'light' fraction (density < 2.0 g $cm^{-3}$) of the > 53 μm soil fraction, which contained the partially decomposed plant litter (henceforth referred to as 'residual plant litter'). Density fractionation of the > 53 μm soil fraction was only done in sandy loam samples amended with maize and oat litter without nutrient solution, due to time constraints.

The original plant litters, whole soil, and the MAOM soil fraction prior to the incubation and at the end of the fourth cycle were analyzed for organic C, total N, $^{13}C$ natural abundance, carbohydrate monomers, and phenol monomers as described below. The residual plant litters collected at the end of the fourth incubation cycle were analyzed only for phenols.

Organic C and N were analyzed with an elemental analyzer interfaced to an isotope ratio mass spectrometer. Carbohydrate monomers were determined according to Martens and Frankenberger, (1990) as adapted by Martens and Loeffelmann (2002). Specifically, soil and plant litter samples were extracted by a sequential hydrolysis of increasing strength, first by adding a weak-acid solution 6 M sulfuric acid ($H_2SO_4$), for 30 min, then diluting to 1 M and autoclaving for 30 min at 121 °C (extract hereafter referred as 'weak-acid solution'). The soil residue was dried overnight and then extracted with a strong-acid solution 18 M $H_2SO_4$ for 30 min, followed by dilution to 1.5 M and autoclaving for 30 min (extract hereafter referred as 'strong-acid solution'). Following centrifuging to isolate the supernatants from residual soil, the pH of both solutions was neutralized with NaOH, then diluted and analyzed through separation by anion exchange chromatography followed by detection through triple pulsed amperometry (Model ICS-5000, Dionex, Sunnyvale, CA). The weak-acid solution was used to extract the monosaccharides fucose, rhamnose, arabinose, galactose, glucose, mannose, and xylose (Chantigny and Angers, 2007). We refer to the sum of the weak-acid extractable monosaccharides as the 'hemicellulose index.' The strong-acid solution is often equated to cellulosic glucose, so glucose in this extract is henceforth referred to as 'cellulose index.', although Chantigny and Angers (2007) caution that some of this glucose can also be of non-cellulosic origin. The ratio of (galactose + mannose)/(arabinose + xylose) was used as to describe the relative significance of microbial versus plant sources for the carbohydrates present in the soil (Cheshire, 1977; Murayama, 1984; Oades, 1984). The summed yield of cellulose- and hemicellulose indices is henceforth referred to as 'total carbohydrates'. The mass of C in total carbohydrates and all single monosacharides was normalized to soil C mass measured in the MAOM soil fraction (i.e., g carbohydrates $kg^{-1}$ soil C).

Phenolic compounds and total lignin were analyzed in whole soil, MAOM, and plant litter samples prior to the incubation study, and residual plant litter at the end of the fourth 46-d incubation cycle. Lignin phenol analyses of residual plant litters were limited to sandy loam amended with maize and oat litters due to time constraints. Lignin phenol content was analyzed before and after the incubation with the alkaline CuO oxidation method following Hedges and Mann (1979) as modified by Filley et al. (2008) and quantified by gas chromatography using a flame ionization detector (GC). Samples were derivatized through silylation with N,O-bis(trimethylsilyl)trifluoracetamide. An absolute recovery standard (3,4-dimethoxybenzoate) was added to the samples during the derivatization process. Ethyl vanillin had been added during the extraction as an internal standard. The total yield of phenolic compounds henceforth referred to as 'total lignin' was defined as the sum of vanillyl- (V), syringyl- (S), and cinnamyl– (C) based lignin derivatives (Hedges and Mann, 1979; Filley et al., 2008). We used ratios of these derivatives as proxies for the extent of lignin decomposition (C/V and S/V) as well as Acid-to-Aldehyde (Ac/Al) such as vanillic acid to vanillin [(Ac/Al)v] and syringic acid to syringealdehyde [(Ac/Al)s]. For instance, higher (Ac/Al)v and (Ac/Al)s ratios suggest greater decomposition (Filley et al., 2008), whereas, lower ratios of C/V and S/V indicate greater lignin decomposition, due to the preferential degradation of cinnamyl and syringyl vs. vanillyl (Otto and Simpson, 2006). As suggested by Hedges and Parker (1976), we did not consider *p*-hydroxyl phenols in our results because they can also be derived from non-lignin sources.

### 2.5. Estimation of the change in soil carbon in the MAOM soil fraction

The change in soil C in the MAOM fraction, from the beginning to the end of the incubation cycles, was measured by two methods. The first method used a two-pool $\delta^{13}C$ isotope ratio mixing model of the MAOM and plant litter to calculate the fraction of C in the MAOM that was derived from plant litter C and the amount of MAOM-C accumulated by multiplying by the total amount of C present in the incubated MAOM ($C_{accum}$):

$$C_{accum} = [(\delta^{13}C \text{ incubated MAOM} - \delta^{13}C \text{ MAOM})/(\delta^{13}C \text{ plant} - \delta^{13}C \text{ MAOM})] \text{ x Total MAOM-C} \quad (1)$$

where $\delta^{13}C$ incubated soil is the $\delta^{13}C$ of MAOM at the end of the 4th cycle of the incubation study, $\delta^{13}C$ soil and $\delta^{13}C$ plant are the $\delta^{13}C$ values of the MAOM and plant litter prior to the incubation, respectively. This was possible because $\delta^{13}C$ varied across plant litters from −11.81 to −30.68 $\delta^{13}C$ and MAOM from −17.73 to −20.60 $\delta^{13}C$ (Table 1). The second method calculated the accumulation of MAOM C and N from the difference in concentration from the beginning to end of the incubation cycles. This method allowed the calculation of accumulated N as well as the C/N ratio of accumulated MAOM. From here forward, the first method is referred as '$\delta^{13}C$ two-pool mixing model', and the second as 'direct measurement.'

We estimated the efficiency of litter-C to MAOM-C transfer as: $C_{min}/(C_{min} + \text{MAOM-}C_{accum})$ where Cmin is the total amount of mineralized litter C across the four 46-d incubations and MAOM-$C_{accum}$ is the total amount of litter-C that accumulated in MAOM during the four 46-d incubations.

### 2.6. Potential N mineralization

To assess potential N mineralization of accumulated MAOM-N (i.e., post-incubation stability), we conducted a short-term anaerobic incubation at the end of the 4th incubation cycle according to Schomberg et al. (2009). After winnowing of residual litter, a 5-g soil subsample was mixed with 12.5 mL of water in a tube to purge all the air and to ensure anaerobic conditions. The temperature was maintained at 40 °C for seven days. At the end of the incubation, samples were extracted with 4 M potassium chloride (KCl). Soil ammonium-N ($NH_4^+$-N) was measured according to Hood-Nowotny et al. (2010). Potentially mineralizable N (PMN) was calculated as the change in the concentration of $NH_4^+$-N during the incubation (i.e., incubated minus initial).

### 2.7. Statistical analysis

Statistical analyzes were performed using SAS software version 9.4 (SAS Institute, Cary, NC, USA). Statistical differences among soil type and plant litter quality with or without nutrient addition were determined using an analysis of variance (PROC GLM), which allowed us to conduct statistical comparisons among treatment means. If the analysis of variance detected a statistical significance at $\alpha = 0.05$ level, Tukey's pair-wise multiple comparison tests were performed to identify

which treatments were significantly different.

## 3. Results

### 3.1. Soil and plant litter pre-incubation

The sandy loam and silty loam soils had similar C concentrations, but very different C/N ratios (Table 1). Relative to the whole soil, the fine mineral soil fraction (i.e., MAOM) had lower $\delta^{13}C$, lower C/N ratio, and greater ratio of microbial-to-plant derived carbohydrates. The MAOM accounted for 47% of whole soil C in the sandy loam soil and 57% of the whole soil C in the silty loam soil.

Maize and soybean litters had higher C/N ratios and lower N concentrations than oat and alfalfa litters (Table 1). The C/N ratios of maize and soybean litters were approximately four-times higher than those of oat and alfalfa litters. The concentration of carbohydrates in plant litters differed from highest to lowest: maize > oats > soybean > alfalfa (Table 1).

### 3.2. Measurement of C accumulation in MAOM: a methods comparison

There was a strong correlation between C accumulation in MAOM as measured by the $\delta^{13}C$ two-pool mixing model and the direct methods ($R = 0.99$, $P < 0.001$; Fig. S2). The slope of the best-fit linear relationship did not differ from 1:1 with or without y-intercept forcing through zero. Thus, from here on, we use the direct method.

### 3.3. Carbon mineralization

Carbon mineralization was affected by litter quality, soil type, and the three-way interaction of these factors with nutrient addition (Table 2). The effect of nutrient addition was small and inconsistent. In contrast, C mineralization differed by 94% across plant litters and 47% across soil types. In contrast, C mineralization differed across nutrient additions by only 2% (Fig. S2). Moreover, neither the interaction between soil type and nutrient addition nor the interaction between plant litter quality and nutrient addition were significant. Therefore, we focused on the two-way interaction between soil type and plant litter quality, which had a large effect on C mineralization (Table 2). Mean cumulative C mineralization from silty loam across all four plant litters was almost double that of sandy loam (5.24 vs. 3.23 g C $kg^{-1}$ whole soil; Table 2). However, mean cumulative C mineralization of both soils incubated with oat and alfalfa litters was almost triple that of soils incubated with maize and soybean litters (6.22 vs. 2.25 g C $kg^{-1}$ whole soil; Table 2).

### 3.4. Carbon and nitrogen accumulation in the MAOM soil fraction

Litter quality had the greatest effect on C accumulation in MAOM per g of litter-C added. Although significant, the effect of nutrient addition and its interaction with soil type were relatively small. The amount of MAOM-C accumulated per unit of litter-C added differed by 46% across litter types, 7% across soil types, and 10% across nutrient additions (Fig. S3). Amendment with oat and alfalfa litters led to greater MAOM-C accumulation per g of litter-C added compared to the maize and soybean litters (0.11 vs. 0.07 g MAOM-C accumulated $g^{-1}$ litter-C addition; Table 2). Nutrient addition reduced C accumulation in MAOM across all litters except alfalfa where it had no effect (Fig. S3). Litter quality also had the greatest effect on N accumulation in MAOM per g of litter-N. Similar to C, the amendment of oat and alfalfa litters led to greater MAOM-N accumulation per g of litter-N added compared to maize and soybean litters.

Although soil type, plant litter quality, and nutrient addition interacted to affect the C/N ratio of accumulated MAOM, litter quality had the greatest effect (Table 2). Similar to C mineralization and C accumulation in MAOM, the effect of nutrient addition on the C/N ratio of accumulated MAOM was small compared to soil type and plant litter quality. The C/N ratio of accumulated MAOM was greater in the silty loam than sandy loam, and decreased in both soil types in the following order: soybean > maize > oats > alfalfa (Table 2).

### 3.5. Carbon mass balance

On a whole soil basis, most of the added plant litter-C was located in the residual plant litter pool that was removed by winnowing at the end of each incubation cycle (neither mineralized nor transferred to the

**Table 2**
Cumulative C mineralization, C and N accumulation in MAOM soil fraction and the C/N ratio of accumulated MAOM at the end of the fourth 46-d aerobic incubation. Data are means of the nutrient treatment (n = 8, ± standard error). Significant differences marked by different case letters ($P < 0.05$). Lower case letters correspond to the interaction of soil type and litter quality, and upper case letters correspond to the effect of plant litter quality.

| Soil | Plant litter | Cumulative C mineralization (g $CO_2$-C $kg^{-1}$ whole soil) | Carbon accumulation (g MAOM-C $g^{-1}$ litter-C addition) | Nitrogen accumulation (g MAOM-N $g^{-1}$ litter-N addition) | C/N ratio of accumulated MAOM |
|---|---|---|---|---|---|
| Sandy loam | | | | | |
| | Maize | 1.68 (0.05)f | 0.07 (0.00)C | 0.26 (0.01)c | 16.6 (0.40)d |
| | Soybean | 0.91(0.02)g | 0.07 (0.00)C | 0.26 (0.01)c | 23.0 (0.38)b |
| | Oats | 5.99 (0.16)b | 0.10 (0.01)B | 0.38 (0.02)b | 5.38 (0.07)e |
| | Alfalfa | 4.35 (0.10)c | 0.12 (0.00)A | 0.41 (0.01)ab | 4.88 (0.11)e |
| | Mean | 3.23 (0.83) | 0.09 (0.01) | 0.33 (0.01) | 12.5 (3.14) |
| Silty loam | | | | | |
| | Maize | 3.77 (0.05)d | 0.06 (0.00)C | 0.20 (0.02)d | 20.5 (0.66)c |
| | Soybean | 2.64 (0.05)e | 0.07 (0.00)C | 0.22 (0.02)d | 26.7 (1.27)a |
| | Oats | 8.71 (0.14)a | 0.09 (0.00)B | 0.38 (0.01)b | 4.76 (0.08)e |
| | Alfalfa | 5.84 (0.05)b | 0.12 (0.00)A | 0.43 (0.02)a | 4.48 (0.08)e |
| | Mean | 5.24 (0.94) | 0.08 (0.01) | 0.31 (0.02) | 14.1 (3.97) |
| **Factor** | | **P-value** | | | |
| Soil type | | < 0.001 | 0.014 | 0.027 | < 0.001 |
| Plant litter | | < 0.001 | < 0.001 | < 0.001 | < 0.001 |
| Nutrient | | 0.065 | < 0.001 | 0.002 | 0.027 |
| Soil type x Plant litter | | < 0.001 | 0.498 | 0.002 | < 0.001 |
| Soil type x Nutrient | | 0.513 | 0.021 | 0.001 | < 0.001 |
| Plant litter x Nutrient | | 0.760 | 0.478 | 0.306 | 0.003 |
| Soil type x Plant litter x Nutrient | | < 0.001 | 0.240 | 0.063 | < 0.001 |

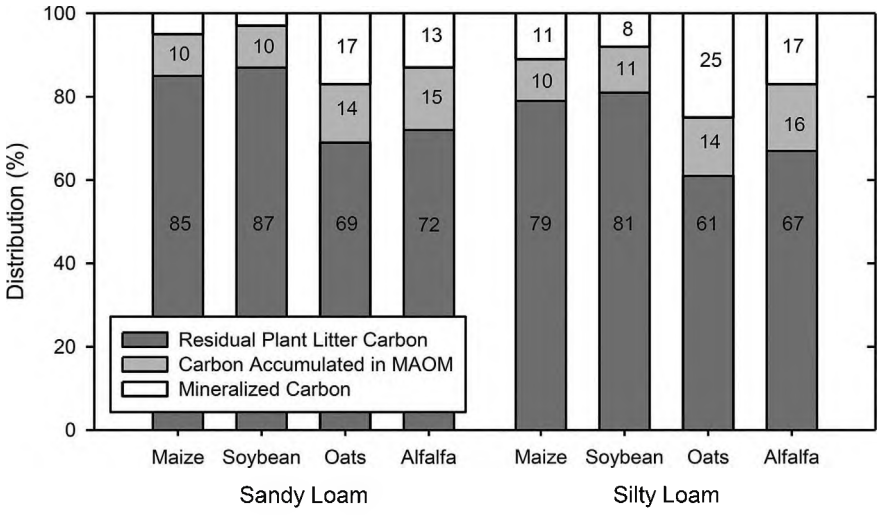


**Fig. 1.** Distribution of litter carbon in three fractions determined at the end of the fourth incubation cycle. Residual plant litter (white bar), MAOM-carbon (light gray bar), and mineralized-carbon (dark gray bar). Data are mean values (n = 8) pooled across the two nutrient addition treatments (without and without nutrient addition) due to the small effect of nutrient addition (Table 2).

MAOM soil fraction). On average, this pool accounted for 75% of the C addition (Fig. 1) despite the fact that the rate of C mineralization at day 46 of the incubation cycles was < 5% of the initial rate and was no longer affected by litter quality (Fig. S2). The amount of removed plant litter differed among soil types: the sandy loam had 8% more residual plant litter-C than did the silty loam (Fig. 1). Maize and soybean litters had greater amounts of residual plant litter-C than did oats and alfalfa. The proportion of plant litter-C accumulated in the MAOM per kg of whole soil was affected by plant litter quality more than by soil type (Fig. 1).

Soils amended with oat and alfalfa litters accumulated more MAOM-C than those amended with soybean and maize litters (Table 2). However, the efficiency of MAOM-C accumulation (g accumulated MAOM-C g mineralized $C^{-1}$) was greater for soybean and maize litters than oat and alfalfa litters (Fig. 2). Although the sandy loam accumulated more MAOM-C per unit of C mineralized compared to the silty loam, the pattern of MAOM-C accumulation across litters was the same in both soil types and nutrient additions. The nutrient addition had a smaller effect on the amount of MAOM-C that accumulated per unit of C mineralized. Across all litters, accumulation of MAOM-C was approximately 11% lower with nutrient addition than with no-nutrient addition (Fig. S5).

### 3.6. Carbohydrates in MAOM

Overall, soils amended with maize and soybean litters had higher concentrations of each monosaccharide and total carbohydrates in MAOM than did the soils amended with oat and alfalfa litters (i.e., g carbohydrates $kg^{-1}$ soil C; Table 3). For example, cellulose indices in both soils amended with maize and soybean litters (86.55 ± 10.59 g $kg^{-1}$ soil C) were higher than soils amended with oat and alfalfa litters (32.97 ± 9.44 g $kg^{-1}$ soil C). Soil type did not significantly affect the cellulose index across all four litters, but this index was numerically greater for maize and soybean in the sandy loam than in the silty loam, especially for soybean (116 vs. 68, respectively; Table 3). The hemicellulose index was only affected by plant litter quality. Soils amended with soybean and maize litter had higher hemicellulose indices than did soils amended with oats and alfalfa (Table 3). Nevertheless, the ratio of microbial-to plant-derived carbohydrates was affected only by plant litter quality and was greater in oats, alfalfa, and soybean than in maize, on average 0.61 vs. 0.39, respectively (Table 3).

### 3.7. Phenols and total lignin content of residual plant litter

Comparisons of lignin at the end of the fourth 46-d incubation were

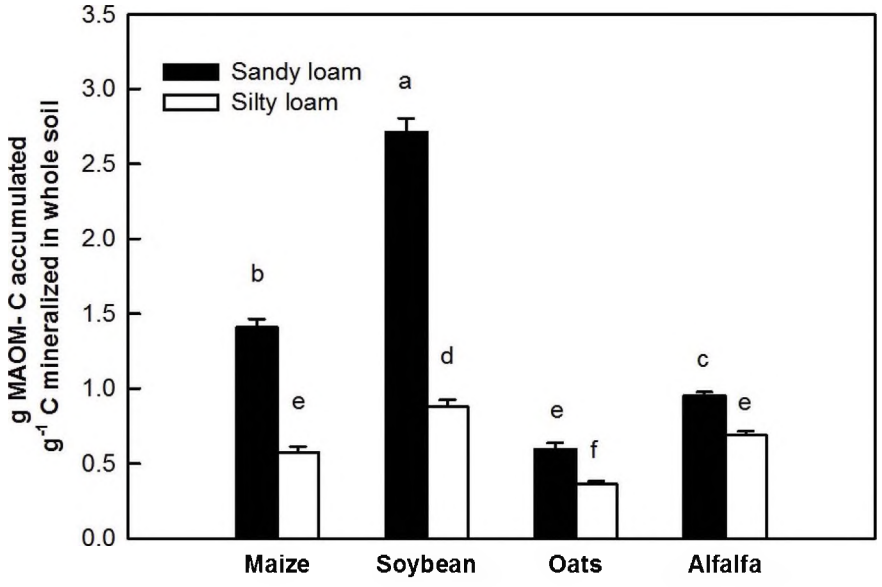


**Fig. 2.** Amount of plant litter-C accumulated in the MAOM soil fraction per unit of C mineralized at the end of the fourth incubation cycle. Data are mean values ± standard error (n = 8) pooled across the two nutrient addition treatments (without and without nutrient addition) due to the small effect of nutrient addition (Table 2). Significant differences between treatments are marked by different lower case letters ($P < 0.001$) according to Tukey's least significant difference test.

**Table 3**
Carbohydrate concentrations of the MAOM soil fraction of samples with no nutrient addition after the four incubation cycles. Lowercase letters indicate the two-way interaction between soil type and plant litter was not significant. Uppercase letters indicate the two-way interaction was significant and thus the letters are comparing means (n = 4, ± standard error) across both litter and soil types (pairwise *t*-test with Tukey-adjustment, $P < 0.05$). The effect of soil type on mannose was higher in silty loam than in the sandy loam samples ($P < 0.05$).

| Soil type | Plant litter | Galactose | Mannose | Arabinose | Xylose | Cellulose Index | Hemicellulose Index | Total Carbohydrates | (galactose + mannose)/(arabinose + xylose)[a] |
|---|---|---|---|---|---|---|---|---|---|
| | | g kg$^{-1}$ soil C | | | | | | g kg$^{-1}$ soil C | |
| Sandy loam | | | | | | | | | |
| | Maize | 35 (1.9)b | 15 (0.7)b | 41 (3.1)a | 61 (9.8)a | 85 (5.6)B | 260 (17.2)a | 345 (18.7)a | 0.42 (0.04)b |
| | Soybean | 51 (2.3)a | 22 (0.5)a | 40 (0.8)b | 57 (3.2)a | 116 (7.4)A | 280 (8.8)a | 396 (11.2)a | 0.63 (0.03)a |
| | Oats | 27 (1.1)c | 13 (0.4)b | 36 (1.4)b | 23 (0.6)b | 13 (4.7)C | 212 (3.4)b | 225 (7.8)b | 0.57 (0.01)a |
| | Alfalfa | 26 (2.7)c | 15 (0.2)b | 27 (2.5)c | 26 (3.5)b | 45 (9.6)C | 169 (16.4)b | 214 (17.3)b | 0.65 (0.03)a |
| Silty loam | | | | | | | | | |
| | Maize | 38 (1.5)b | 17 (1.3)b | 47 (1.8)a | 81 (2.9)a | 76 (3.5)B | 317 (7.4)a | 393 (9.5)a | 0.36 (0.02)b |
| | Soybean | 50 (4.9)a | 26 (2.4)a | 38 (3.6)b | 59 (5.5)a | 68 (3.0)B | 314 (16.3)a | 382 (11.1)a | 0.64 (0.01)a |
| | Oats | 28 (2.2)c | 16 (1.2)b | 36 (2.9)b | 26 (3.2)b | 20 (1.0)C | 216 (10.1)b | 237 (10.9)b | 0.60 (0.02)a |
| | Alfalfa | 21 (9.8)c | 17 (1.9)b | 29 (4.2)c | 28 (3.6)b | 52 (4.9)C | 164 (17.5)b | 217 (11.9)b | 0.55 (0.13)a |
| **Factor** | | **P-value** | | | | | | | |
| Soil type | | 0.868 | < 0.01 | 0.316 | 0.055 | 0.146 | 0.193 | 0.495 | 0.330 |
| Plant litter | | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 |
| Soil type x Plant litter | | 0.724 | 0.966 | 0.498 | 0.208 | 0.036* | 0.581 | 0.649 | 0.437 |

[a] The ratio of microbial-vs. plant-derived carbohydrate was calculated as: (galactose + mannose)/(arabinose + xylose) as suggested by Cheshire (1977), Murayama (1984) and Oades (1984). Ratios around 0.5 suggest mostly plant origin whereas ratios around 2 suggest mostly microbial origin (Palazzo et al., 2008).

**Table 4**
Mean concentrations and molecular parameters of lignin phenols in the pre- and post-incubation mineral-associated organic matter and plant residues prior to incubation, and at the end of 4th 46-d aerobic incubation (n = 4). Values followed by a different letter are significantly different at $P > 0.05$.

| Type | Vanillyl (V) | Syringyl (S) | Cinnamyl (C) | Total Lignin | C/V | S/V | C/SVC | Ac/Al$_{(v)}$[a] | Ac/Al$_{(s)}$[b] |
|---|---|---|---|---|---|---|---|---|---|
| **Plant litter (g phenol kg$^{-1}$ plant litter)** | | | | | | | | | |
| Maize | 9.65 | 11.3 | 17.2 | 38.2 | 1.8 | 1.2 | 0.45 | 0.14 | 0.29 |
| Oats | 4.93 | 2.74 | 4.89 | 12.6 | 0.99 | 0.56 | 0.39 | 0.31 | 0.72 |
| **Pre-incubation mineral-associated organic matter (g phenol kg$^{-1}$ soil < 53 μm)** | | | | | | | | | |
| Sandy loam | 0.06 | 0.06 | 0.03 | 0.15 | 0.41 | 0.94 | 0.17 | 0.11 | 0.71 |
| **Post-incubation mineral-associated organic matter (g phenol kg$^{-1}$ soil < 53 μm)** | | | | | | | | | |
| Sandy loam + Maize | 0.1 | 0.04 | 0.06 | 0.2 | 0.63 | 0.39 | 0.31 | 1.7 | 7.05 |
| Sandy loam + Oats | 0.08 | 0.05 | 0.03 | 0.16 | 0.41 | 0.63 | 0.2 | 0.8 | 2.12 |
| **Post-incubation residual plant litter (g phenol kg$^{-1}$ plant litter)** | | | | | | | | | |
| Sandy loam + Maize | 2.87 | 4.15 | 5.23a | 12.3a | 1.85a | 1.43a | 0.43a | 0.13 | 0.38b |
| Sandy loam + Oats | 2.49 | 2.59 | 1.72b | 6.80b | 0.69b | 1.04b | 0.25b | 0.15 | 0.69a |

[a] ratio of vanillic acid to vanillin.
[b] ratio of syringic acid to syringealdehyde.

limited to maize and oats in the sandy loam soil. The total amount of lignin (i.e., the sum of syringyl, cinnamyl, vanillyl) in residual plant litter collected by density floatation at the end of the fourth incubation cycle, and the comparison among phenol monomers (e.g., C/V, S/V, C/SVC, Ac/Al(s), were all affected by plant litter quality (Table 4). For example, the total lignin content measured in residual maize litter was two times higher than residual oat litter (12.25 versus 6.80 g phenol kg$^{-1}$ residual plant litter respectively; Table 4). Moreover, the C/V, S/V and C/SVC ratios ($P < 0.05$ for all) were higher in maize compared to oat litter (Table 4), whereas, the Ad/Al(s) ratio ($P < 0.05$) was higher in oats compared to maize litter. Compared to the pre-incubation MAOM, total lignin of the post-incubation MAOM increased numerically more with maize addition than with oats, due to greater accumulation of the V and C phenols.

### 3.8. Potential nitrogen mineralization

Amendment of oat and alfalfa litters led to 67% more accumulation of MAOM-N than did ammendment of maize and soybean litters averaged across both soils (Table 2). However, after winnowing of residual litter, a greater proportion of accumulated MAOM-N in soils amended with oat and alfalfa litters was mineralizable within 7 d than in soils amended with maize and soybean litters (0.19–0.06 mg N mineralized mg$^{-1}$ accumulated MAOM-N; Fig. 3). The amount of potentially mineralizable N per amount of N accumulated in the MAOM soil fraction (i.e., mg N mineralized mg$^{-1}$ MAOM-N accumulated) was affected by soil type, plant litter quality and its interaction with nutrient-addition ($P < 0.05$). Per unit of MAOM-N accumulated, sandy loam mineralized more N than did silty loam with or without nutrient addition (0.15 vs. 0.11 mg N mineralized mg$^{-1}$ MAOM-N accumulated). Similar to C mineralization and accumulation in MAOM, the effect of nutrient addition on mineralization of accumulated MAOM-N was significant yet small; nutrient addition led to slightly less N mineralization (0.13 vs. 0.12 mg N mineralized mg$^{-1}$ MAOM-N accumulated).

## 4. Discussion

Plant litter quality can have a large effect on SOM stabilization, yet

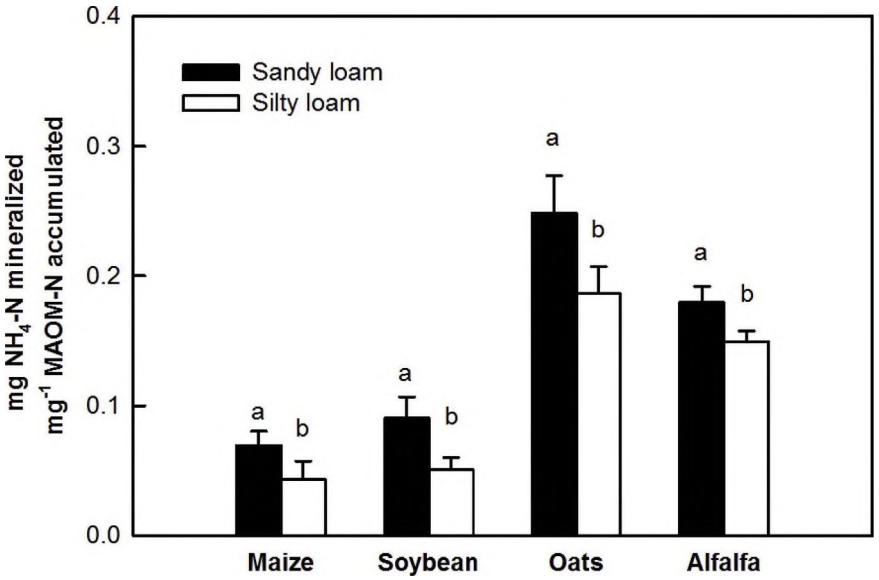


**Fig. 3.** Potentially mineralizable nitrogen at the end of the fourth 46-day incubation after winnowing of residual litter addition. Mean values (n = 8, ± standard error) reported for each plant litter and soil type combination averaged across the nutrient treatments. Significant differences between soil types are marked by different lower case letters ($P < 0.05$) according to Tukey's least significant difference test.

there is great uncertainty about how litter quality interacts with microbial activity to control SOM stabilization. Although well-developed theory predicts that litters with high mineralization rates (high quality) lead to greater SOM stabilization in the mineral soil matrix than do litters with low mineralization rates (low quality), there is no consistent effect of litter quality on SOM stabilization (Castellano et al., 2015). We conducted a highly controlled experiment to help determine how litter quality affects C flows and SOM stabilization in the early phases of decomposition when litter quality has the greatest effect on decomposition rate.

Although high quality litters led to greater accumulation of MAOM, they also released more $CO_2$. Consequently, the efficiency of MAOM accumulation was lower for high quality litters (Fig. 2). This result is partly consistent with the "Microbial Efficiency-Matrix Stabilization (MEMS)" framework (Cotrufo et al., 2013). High quality litters led to greater litter-C transfer to the mineral soil matrix (Table 2), but not when normalized by litter-C mineralization (Fig. 2).

Traditional ecological concepts suggest low quality litters with slow decomposition rates promote the accumulation of plant compounds in SOM that are inherently chemically resistant to decomposition (Prescott, 2010). In contrast, later observations led to a new conceptual model of SOM stabilization that emphasized the role of microbial growth and activity. Initially, this model hypothesized that the role of microbes in SOM stabilization was limited to a single pathway where the production of microbial necromass drives the accumulation of MAOM (Grandy and Neff, 2008) and, as a result, high quality litters lead to greater accumulation of MAOM because they maximize microbial growth and activity (Cotrufo et al., 2013). As a corollary, these concept models also predicted that the chemical composition of MAOM should be similar across different litter chemistries because the SOM stabilized in MAOM passes through a 'microbial filter' (Cotrufo et al., 2013; Grandy and Neff, 2008; Melillo et al., 1989). Our observation of greater MAOM accumulation in soils amended with relatively high quality oat and alfalfa litters are partly consistent with this prediction.

However, high quality litters in our study actually had less MAOM accumulation per g of respired C (Fig. 2), perhaps reflecting that MAOM accumulation was largely driven by differences in the rates of litter depolymerization across litter types rather than differences in the fate of depolymerized litter and its utilization by microbes. Further, this result suggests that microbial C use efficiency (CUE) in our experiment might not have been higher during microbial metabolism of the higher quality litters, a central tenet of the MEMS framework (Cotrufo et al., 2013). Alternatively, the lower efficiency of MAOM accumulation from higher quality litters (g MAOM accumulation $g^{-1}$ of respired C) might occur if higher quality litters lead to greater mineralization of microbial biomass and byproducts that were initially derived from plant litter, but then recycled prior to accumulation in MAOM. In other words, even if the microbial CUE of plant substrates is higher for high quality litters, faster turnover of those microbial communities or tighter internal recycling of microbial necromass could lead to proportionally lower retention of litter-derived C over time.

More recently, the hypothesized role for microbial metabolism in SOM stabilization has been expanded beyond the microbial filter hypothesis to include two distinct pathways for the stabilization of plant- and microbial-derived molecules. Liang et al. (2017) categorize these pathways as *ex vivo* modification and *in vivo* turnover. Codification of these pathways offers new opportunities to explain the direct stabilization of plant-derived compounds in MAOM as well the inconsistent effect of litter quality on SOM stabilization. In the *ex vivo* modification pathway, extracellular enzymes release depolymerized products of plant compounds that are not readily assimilated by microbes and thus susceptible to rapid stabilization in MAOM. In contrast, in the *in vivo* turnover pathway, microbes assimilate plant-derived organic matter and synthesize new organic C-compounds that are subsequently stabilized in the mineral soil matrix. Although *in vivo* turnover appears to be the dominant pathway in many soils, there is empirical evidence for the existence of both pathways (Angst et al., 2017; Gillespie et al., 2014; Miltner et al., 2012).

In this study, large differences in C/N ratio of accumulated MAOM (Table 2) provide strong support for the existence of both stabilization pathways, suggesting that litter quality can control the relative importance of each stabilization pathway as well as the chemical composition and stability of MAOM. The low-quality maize and soybean litters produced MAOM with a C/N ratio > 20, which is indicative of a direct plant source. In contrast, the high-quality oat and alfalfa litters produced MAOM with a C/N ratio < 5, which is indicative of a microbial source (Table 2). Carbohydrate and lignin data were largely consistent with these generalizations. Although ratios of microbial- and plant-derived carbohydrate monomers suggested most carbohydrates in MAOM were plant-derived, the MAOM in maize-incubated soils was significantly more plant-derived than other litters (Table 3). Moreover, cellulose and hemicellulose indices as well as total carbohydrates of post-incubation MAOM were greater for low quality maize and soybean than high quality oats and alfalfa. These differences occurred despite the fact that oats and alfalfa treatments accumulated more MAOM than maize and soybean albeit less efficiently. Although our comparison of

post-incubation lignin concentrations in MAOM was limited to sandy loam soils incubated with low quality maize and high quality oat litter, these data indicate that lignin was an important contributor to MAOM in the maize treatment (Table 4). Together, these patterns complement previous research that demonstrates MAOM can represent a diverse pool of organic compounds, and the source of MAOM can differ with litter chemistry (Gillespie et al., 2014; Quideau et al., 2001; Stewart et al., 2011).

In addition to the direct stabilization of plant biomolecules on mineral surfaces, microbial-derived extracellular polysaccharides (EPS) could contribute directly or indirectly to the biomolecules we measured in MAOM. The microbial EPS could be chemically stabilized on mineral surfaces or promote physical protection of plant biomolecules within mineral particles (i.e., aggregation) that we were unable to disperse (Chenu, 1993). Similarly, inorganic N could contribute to the N we measured in MAOM (Christensen and Schjonning, 2004). However, measurements of $NH_4^+$ pools in 2M KCl extracts for potential N mineralization assays indicate that the $NH_4^+$ pool in MAOM was small (< 5% of accumulated MAOM-N) and did not consistently differ across litter types (data not shown). Moreover, less than 12% of the accumulated MAOM-N was potentially mineralizable.

Nevertheless, despite the low potential mineralization of accumulated MAOM-N, relative differences in potential N mineralization across litter types were consistent with the differences in MAOM chemistry and concentration across litter types, leading us to reject the hypothesis that MAOM would be similarly resistant to mineralization across all litter types. The MAOM-N that accumulated in maize and soybean treatments had lower potential N mineralization (both absolute and per mass of accumulated MAOM-N; Fig. 3). Given the different C/N stoichiometries and biomolecular compositions of accumulated MAOM in maize and soybean vs. oat and litter treatments, this pattern is consistent with well-known controls on litter decomposition and N mineralization independent from the mineral soil matrix: litters with relatively high C/N ratios and fractions of structural compounds decompose relatively slowly (Parton et al., 2007).

With the exception of alfalfa, the treatments that received nutrient additions accumulated less MAOM (Fig. S4 & S5). These results extend microbial 'N mining' theory (Moorhead and Sinsabaugh, 2006) from litter decomposition to the stabilization of MAOM. Despite the wide range of litter C/N ratios and biomolecular composition across oats, maize and soybean treatments (Tables 2–4), nutrient addition consistently suppressed the accumulation of MAOM. In these nutrient poor soils, it is likely that microbes respond to nutrient limitation by increasing the mineralization of litter in search of nutrients rather than energy; in contrast, when nutrients are sufficient, microbes do not decompose relatively low- or zero-energy substrates (Craine et al., 2007; Moorhead and Sinsabaugh, 2006). Thus, the suppression of litter depolymerization may have suppressed the accumulation of MAOM. We hypothesize that the suppressive effect of nutrient addition on SOM stabilization in the mineral soil matrix may be most important in soils where *ex vivo* modification (i.e., depolymerization of plant biomolecules) is a significant contributor to MAOM; this could explain why nutrient additions did not influence MAOM accumulation with amendment by high-quality alfalfa, which probably led to MAOM formation predominately via the in *vivo* pathway.

Thus, the occurrence of microbial N mining may provide insight about the relative importance of *ex vivo* modification and *in vivo* turnover pathways to SOM stabilization. Nutrient addition has been shown to increase, decrease or have little effect on the accumulation of MAOM (Bradford et al., 2013; Creamer et al., 2014; Gentile et al., 2011; Gillespie et al., 2014; Kirkby et al., 2013). In soils with low SOM stocks, nutrient addition may reduce the absolute amount and relative importance of *ex vivo* modification such that microbes do not depolymerize plant structural compounds and thus these compounds are not stabilized in MAOM. In contrast, in soils with high SOM stocks and large pools of available energy, the addition of nutrients may increase microbial biomass and turnover, thus increasing the absolute amount and relative importance of *in vivo* turnover. Interactions between litter quality and the indigenous pool of MAOM may control the outcome.

## 5. Conclusions

Litter quality had a strong effect on MAOM accumulation that was consistent across soil type and nutrient addition. However, we observed several unexpected effects of litter quality on MAOM accumulation. Litters with rapid mineralization rates (i.e., high quality) led to greater, but less efficient accumulation of MAOM than litters with slow mineralization rates (i.e., low quality, Figs. 1 and 2). Moreover, the MAOM that accumulated from litters with rapid mineralization rates was less stable than MAOM that accumulated from litters with slow mineralization rates (Fig. 3). Plant-derived carbohydrates were significant contributors to MAOM (Table 3), adding to a growing number of reports that demonstrate MAOM often contains substantial amounts of plant biomolecules (Angst et al., 2017a,b; Cotrufo et al., 2015; Gillespie et al., 2014; Sanderman et al., 2014). Greater accumulation of MAOM in soils without nutrient addition suggest plant biomolecules may be particularly important contributors to MAOM in nutrient-poor environments where microbes depolymerize large amounts of plant compounds in search of nutrients.

## Acknowledgements

This work was funded by the Iowa State University Plant Sciences Institute Faculty Scholar Program, the United States Department of Agriculture National Institute of Food and Agriculture Foundational programs (grant number 2014-67019-21629) and the Secretaria de Educacion Superior, Ciencia, Tecnologia e Innovacion from the Ecuadorian Government. We are grateful to Terry Grimard and Jordan Kersey for assistance with laboratory analyses.

## Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.soilbio.2018.07.010.

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