Pathways of soil organic matter formation from above and belowground inputs in a *Sorghum bicolor* bioenergy crop

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**Abstract**
When aboveground materials are harvested for fuel production, such as with *Sorghum bicolor*, the sustainability of annual bioenergy feedstocks is influenced by the ability of root inputs to contribute to the formation and persistence of soil organic matter (SOM), and to soil fertility through nutrient recycling. Using $^{13}$C and $^{15}$N labeling, we traced sorghum root and leaf litter-derived C and N for 19 months in the field as they were mineralized or formed SOM. Our in situ litter incubation experiment confirms that sorghum roots and leaves significantly differ in their inherent chemical recalcitrance. This resulted in different contributions to C and N storage and recycling. Overall root residues had higher biochemical recalcitrance which led to more C retention in soil (27%) than leaf residues (19%). However, sorghum root residues resulted in higher particulate organic matter (POM) and lower mineral associated organic matter (MAOM), deemed to be the most persistent fraction in soil, than leaf residues. Additionally, the overall higher root-derived C retention in soil led to higher N retention, reducing the immediate recycling of fertility from root as compared to leaf decomposition. Our study, conducted in a highly aggregated clay-loam soil, emphasized the important role of aggregates in new SOM formation, particularly the efficient formation of MAOM in microaggregate structures occluded within macroaggregates. Given the known role of roots in promoting aggregation, efficient formation of MAOM within aggregates can be a major mechanism to increase persistent SOM storage belowground when aboveground residues are removed. We conclude that promoting root inputs in *S. bicolor* bioenergy production systems through plant breeding efforts may be an effective means to counterbalance the aboveground residue removal. However, management strategies need to consider the quantity of inputs involved and may need to support SOM storage and fertility with additional organic matter additions.

**KEYWORDS**
aggregates, biofuel crops, litter decomposition, physical fractionation, roots, shoots, soil organic matter, sorghum

**Abbreviations:** ADF, acid detergent fiber; HWE, hot water extractable; LCI, lignocellulose index; LDC, litter derived carbon; LDN, litter derived nitrogen; LF, light fraction; MAOM, mineral associated organic matter; M, macroaggregates; m, microaggregates; Mm, microaggregates inside macroaggregates; POM, particulate organic matter; SOM, soil organic matter.
The sustainability of bioenergy feedstocks has long been debated (Fargione, Hill, Tilman, Polasky, & Hawthorne, 2008; Karp & Shield, 2008; Searchinger et al., 2008) prompting extensive research to focus on the ability of specific bioenergy feedstocks to sequester carbon (C) in the soil in order to offset the production of greenhouse gases throughout the lifecycle of the feedstock (Heaton, Dohleman, & Long, 2008; Lemus & Lal, 2005; Nocentini, Field, Monti, & Paustian, 2017). *Sorghum bicolor* has been identified as an important bioenergy feedstock due to the combination of several unique characteristics, such as drought tolerance, high water use efficiency, low nutrient requirements, vast genetic diversity, deep root systems, and tremendous yield potential (up to 30 T per ha) (Bean, Blumenthal, Rooney, & Mullet, 2007; Mullet et al., 2014; Olson et al., 2013; US Department of Energy, 2016). Given the vast genetic diversity of sorghum, it can contribute to bioenergy production in three ways: sorghum grains from grain varieties for first generation biofuel production; crop residues remaining after grain harvest for cellulosic biofuel production; and as a dedicated energy crop—high biomass varieties or high sugar varieties’ aboveground materials—for cellulosic biofuel production (US Department of Energy, 2016). In cellulosic biofuel production, aboveground residues are harvested. Thus, aboveground residue C and N are exported out of the system which may be detrimental to soil C stocks and recycling of nutrients in these annual cropping systems (Blanco-Canqui & Lal, 2007; Lal, 2005, 2008). There is a need to understand the ability of cellulosic biofuel crops to contribute to C sequestration while supporting nutrient cycling through the decomposition of below ground organic matter.

Crop research has begun to focus on the enhancement of root systems, such as increases in total root production or specific root length (Kell, 2011, 2012) as a strategy to improve soil health, increase drought tolerance, and as a C sequestration strategy through the contribution of root biomass to soil organic matter (SOM) formation (Paustian, Campbell, Dorich, Marx, & Swan, 2016). Sorghum is a crop of particular interest in this context, because cultivars produce large deep root systems, to 1–2 m in depth (Monti & Zatta, 2009; Sainju, Singh, & Whitehead, 2005; Schittenhelm & Schroetter, 2013; Stone, Goodrum, Nor Jaafer, & Khan, 2001). Additionally, it is unique as an actively researched bioenergy feedstock, because it is grown as an annual crop, thus the entire root system is left to decompose every year. Sorghum root biomass estimates range from ~0.5 to 6.1 mg dry matter per ha based on field measurements, to 7 mg dry matter per ha based on modeled projections (Meki, Snider, Kiniry, Raper, & Rocateli, 2013; Monti & Zatta, 2009; Sainju, Whitehead, & Singh, 2005; Schittenhelm & Schroetter, 2013).

The ability of crop residues to contribute to SOM formation has traditionally focused on aboveground biomass, or shoots. However, live and dead roots are the major actors in the formation, stabilization, and destabilization of SOM (Sokol, Kuebbing, Karlsen-Ayala, & Bradford, 2018), and their contribution to SOM formation has started to receive significant attention (Balesdent & Balabane, 1996; Kong & Six, 2010; Lavallee, Conant, Paul, & Cotrufo, 2018; Rasse et al., 2006; Schmidt et al., 2011). Yet, we lack a complete understanding of how root vs shoot detritus form SOM into differently protected physical fractions, and contribute to soil C and N storage and/or recycling. While the point of entry of plant inputs may significantly determine their fate (Mitchell et al., 2016, 2018; Sokol, Sanderman, & Bradford, 2019), differences in litter chemistry between roots and shoots may be the most important determining factor in their contribution to SOM and the likelihood that this new SOM will persist in the soil (Bird & Torn, 2006). Lavallee et al., 2018).

Plant inputs have been proposed to contribute to SOM formation via two main pathways (Cotrufo et al., 2015). Leaching of water soluble compounds happens early in the decomposition process and leads to the formation of mineral associated organic matter (MAOM) either through direct association with minerals or after being metabolized by microbes (Liang, Schimel, & Jastrow, 2017). Later in the decomposition process, the remaining residue structural components fragment and contribute to SOM mostly in the form of light organic matter (LF) (Haddix, Paul, & Cotrufo, 2016). Further decomposition of plant residues or LF in the soil may also contribute to sand-sized heavy particulate organic matter (POM) formation (Grandy & Neff, 2008). LF, heavy POM and MAOM can be considered primary SOM fractions that may be found in soil free or occluded in aggregates of different size classes (Christensen, 2001). Aggregates are composite dynamic structures (Jastrow, 1996; Poeplau et al., 2018; Six, Elliott, & Biochemistry, 2000). Micro-aggregates (250–53 μm) have been consistently found to resist disturbance, thus are believed to offer protection from further mineralization to the occluded primary SOM fractions (Six & Paustian, 2014). The role of organic matter in the formation of aggregates is well studied, but much less is known regarding the role of aggregates in the formation of new SOM and its distribution between different primary fractions (Jastrow, 1996; Six, Elliott, et al., 2000).

Mineral association and physical occlusion are believed to be the main drivers of long-term SOM persistence (Kleber & Lehmann, 2015), along with environmental inhibition of microbial activity, while biochemical recalcitrance has been dismissed from this role (Dungait, Hopkins, Gregory, & Whitmore, 2012; Marschner et al., 2008; Schmidt et al., 2011). Although biochemical recalcitrance may not correlate to long-term SOM persistence (Kleber & Johnson, 2010; Klotzbücher, Kaiser, Guggenberger, Gatzek, & Kalbitz,
2011; Preston, Nault, & Trofymow, 2009), plant input chemistry determines annual-scale litter decomposition rates (Adair et al., 2008; Preston et al., 2009) and the pathways and efficiency of SOM formation (Cotrufo et al., 2015; Lavalle et al., 2018). Measures of litter chemistry such as hot water extractable C (%HWE-C), lignin content (Melillo, Aber, & Muratore, 1982), C:N ratio (Manzoni, Taylor, Richter, Porporato, & Ågren, 2012), and lignocellulose index (LCI) (lignin/[lignin+α-cellulose]) (Osono & Takeda, 2005) are good indicators of dissolved organic C production (Soong, Parton, Calderon, Campbell, & Cotrufo, 2015), litter mass loss rates, and microbial carbon use efficiency (Manzoni et al., 2012; Moorhead, Lashermeres, Sinsabaugh, & Weintraub, 2013). According to the microbial efficiency-matrix stabilization framework plant inputs characterized by low biochemical recalcitrance (i.e., high %HWE and low C:N and LCI) are expected to contribute to MAOM formation more than biochemically recalcitrant plant inputs (Cotrufo, Wallenstein, Boot, Denef, & Paul, 2013). As an extension, biochemically recalcitrant inputs would preferentially accumulate as POM (Castellano, Mueller, Olk, Sawyer, & Six, 2015). While this hypothesis has been tested and often confirmed in the laboratory (Córdova et al., 2018; Haddix et al., 2016; Lavalle et al., 2018) field experiments are still limited and largely performed in natural forest or grassland systems (Bird, Kleber, & Torn, 2008; Bird & Torn, 2006; Sokol et al., 2018; Soong et al., 2016). Understanding how differences in litter chemistry impact the decomposition and resultant SOM formation of root vs shoots is going to be critical to informing agricultural management, particularly in bioenergy production systems.

We designed a field experiment to address the knowledge gaps highlighted above. Specifically, we compared differences in litter chemistry between S. bicolor roots and leaves to study: (a) how they form SOM into differently protected physical fractions, and contribute to soil C and N storage and/or recycling to depth, and (b) the role of aggregates in SOM formation. We hypothesize that the root residues, because of their higher biochemical recalcitrance will decompose slower, and will contribute more to LF than leaves, which conversely would decompose faster and form relatively more MAOM. Due to the greater LF contribution from roots we expect relatively more N to be released from root than leaf litter over time, and overall relatively more residue-derived N to be accumulated at depth than C from either litter type. Moreover, we expect aggregates to promote MAOM formation, particularly in microaggregates through high physical proximity of the new organic matter to soil minerals.

To test these hypotheses, we incubated $^{13}$C and $^{15}$N enriched sorghum roots or leaves in unique soil-biomass microcosms in situ for 19 months. To test the effect of litter chemistry both litter types were incubated within the same soil volume (i.e., top 0–30 cm). We depicted differences in the chemistry of the litters by C:N, % hemicellulose, % α-cellulose, % acid unhydrolyzable residue (AUR), and % HWE-C.

Incubated soils were destructively harvested and separated into light (LF), heavy POM, and MAOM, as found free or occluded in macro- and micro-aggregates.

2 | MATERIALS AND METHODS

2.1 | Site description

The study was conducted at the Agricultural Research Development and Education Center (ARDEC), Colorado State University, Fort Collins, Colorado (40°39′N, 104°59′W; 1,554 m above sea level). The field site has a history of mixed-use irrigated agriculture. An area of approximately 400 m$^2$ was tilled and planted with S. bicolor v BTx 623 in 2013 and 2014 and irrigated during the growing season for the purpose of this experiment. Average annual precipitation for the area is 330 mm and mean monthly temperature ranges from 0°C in January to 22°C in July. The clay loam soil is classified as a mixed, superactive, mesic, Aridic Haplustalf. Average soil bulk density (BD) ($n = 4$), is 1.07, 1.09, 1.13 g/cm at 0–30, 30–60, and 60–90 cm depths, respectively. Soil texture across the profile is on average ($n = 3$) 34.34% sand, 34.22% silt, and 30.75% clay (percentage sand increased by 52.23% with depth from 29.43% at 0–30 cm to 44.81% at 60–90 cm, but the soil remains within clay loam classification throughout its profile).

2.2 | Soil collection

In October 2013, after sorghum harvest, 36 soil cores up to 90 cm were extracted using a truck mounted hydraulic soil probe (Giddings Machine Company Inc.) approximately 1 m apart throughout the experimental area. A PVC tube was inserted in each hole, as a placeholder, until microcosms were incubated in the field as described below. Each core was divided into three depths: 0–30, 30–60, and 60–90 cm. Soils from each depth were carefully sieved to avoid breaking up soil aggregates, with large rocks and roots removed. The sieved soil was combined and homogenized in large bins according to depth, and kept moist at room temperature for 2 weeks until used for the incubation as described below.

2.3 | Isotopically labeled Sorghum litter production

Sorghum bicolor v. BTx 623 was grown in a $^{13}$C and $^{15}$N continuous labeling chamber as described in Soong et al. (2014). After 22 weeks of growth in the chamber, the plants were harvested. Aboveground plant material was
cut at the base of the plant ~3 cm from the soil surface, separated into leaves and stalks by stripping the leaves from the stalks by hand, and air-dried. Belowground plant material was removed from the potting mixture (sand, vermiculite, and profile porous ceramic) by dumping the whole pots over a 6 mm sieve and gently shaking the roots loose. Roots were rinsed thoroughly with deionized water and air-dried. While *Sorghum* stalks are likely the dominant type of above ground residue after grain is harvested, leaves were used in our study. During preliminary analysis of the litter, leaves and stalks did not differ significantly in their C:N, LCI, and %AUR, but both did differ from roots. HWE-mass was higher for stalks (44.10%; \( SE = 0.97 \)) than leaves (27.01%; \( SE = 0.17 \)) but both were greater than roots (14.69%; \( SE = 0.27 \) \( n = 3 \)). These analyses were repeated for leaves and roots only as reported in the results (Table 1). We concluded that leaves were a good representative of aboveground materials from sorghum in regards to litter chemistry, contrasted well with the litter chemistry of roots, and could be incorporated into the soil more similarly to roots than stalks allowing for a more direct comparison of the two different litters in the sorghum production system. Fine roots, <2 mm in diameter, or leaves, cut into ~2 cm long pieces, were homogenized.

### 2.4 | Field experiment

To study litter decomposition in the field and track litter derived C and N in SOM and along the soil profile a microcosm approach was used, using 1 m long, 5.08 cm diameter PVC tubes. To allow root ingrowth 18 circular holes (3.2 cm diameter) were cut along each of the PVC tubes. Nylon mesh (1.6 mm) was sewn into 1 m columns with

| Table 1 | Carbon (C) and nitrogen (N) concentration and their isotopic composition in bulk soil, native litter, and all the SOM physical fractions isolated in this study, as well as the relative contribution of each fraction to the bulk soil, for the 0–30 cm depth layer. C and N concentrations are averaged across all litter types, harvests and replicates, \( n = 36 \) with ± standard error in parentheses. \(^{13}C\) and \(^{15}N\) isotope delta values are averaged across four replicates and three harvests from the control microcosms, \( n = 12 \) with ± standard error in parentheses.

| Fraction          | Abbreviation | % of total soil | % C  | % N  | \(\delta^{13}C\) | \(\delta^{15}N\) |
|-------------------|--------------|-----------------|------|------|----------------|-----------------|
| Bulk soil         |              | 100             | 2.09 (0.02) | 0.17 (0.003) | −14.17 (0.11) | 6.07 (0.15) |
| Litter residues   |              | 0.03 (0.01)     | 35.76 (0.84) | 1.21 (0.07) | −16.42 (1.71) | 8.47 (2.39) |
| Macroaggregates M | M            | 63.00 (1.01)    | 2.29 (0.01) | 0.19 (0.001) | −14.51 (0.07) | 6.05 (0.13) |
| Free microaggregates m | m | 30.06 (0.82) | 1.53 (0.03) | 0.12 (0.002) | −14.27 (0.20) | 5.61 (0.19) |
| Free MAOM         | MAOM         | 6.55 (0.22)     | 2.37 (0.02) | 6.51 (0.28) | −13.05 (0.16) | 6.51 (0.28) |
| Coarse POM        | Coarse POM   | 3.11 (0.12)     | 1.21 (0.05) | 0.08 (0.004) | −18.02 (0.92) | 5.28 (0.74) |
| Occluded microaggregates M_m | M_m | 36.07 (0.74) | 2.16 (0.05) | 0.19 (0.004) | −15.34 (0.08) | 6.04 (0.26) |
| Occluded MAOM M_MAOM | M_MAOM | 23.82 (0.37) | 2.38 (0.02) | 0.18 (0.002) | −13.38 (0.11) | 6.67 (0.26) |
| Free microaggregate occluded LF m_LF | m_LF | 0.07 (0.02) | 35.67 (0.80) | 2.60 (0.06) | −23.82 (0.70) | 9.24 (1.43) |
| Free microaggregate occluded POM m_heavy POM | m_heavy POM | 12.90 (0.37) | 0.38 (0.01) | 0.019 (0.001) | −11.81 (0.32) | 1.66 (0.22) |
| Free microaggregate occluded MAOM m_MAOM | m_MAOM | 16.86 (0.53) | 1.98 (0.03) | 0.19 (0.002) | −16.15 (0.19) | 6.60 (0.09) |
| Occluded microaggregate LF Mm_LF | Mm_LF | 0.13 (0.02) | 32.18 (0.10) | 2.47 (0.09) | −23.07 (0.09) | 6.18 (0.53) |
| Occluded microaggregate POM Mm_heavy POM | Mm_heavy POM | 10.44 (0.25) | 0.87 (0.02) | 0.07 (0.002) | −17.49 (0.20) | 3.30 (0.15) |
| Occluded microaggregate MAOM Mm_MAOM | Mm_MAOM | 23.68 (0.78) | 2.20 (0.02) | 0.23 (0.003) | −16.36 (0.13) | 6.68 (0.09) |
heavy-duty nylon thread, and placed inside the PVC tubes. Collected soils were then placed at their corresponding field depth (0–30, 30–60 or 60–90 cm) within one continuous soil column. The appropriate weight of soil was used at each depth in order to recreate field site BD. In order to study the effect of root and leaf residue chemistry on their decomposition and resultant SOM formation, isotopically enriched sorghum root or leaf residues (~1 g) were mixed with the 0–30 cm soil depth prior to being funneled into the mesh sock inside the PVC column. Soil without litter was placed at the 30–60 and 60–90 cm depth. Control columns did not receive any litter addition, throughout the depth layers. The mesh column was closed at both ends with a zip tie. The in situ decomposition study began on November 9, 2013, when 36 soil-biomass microcosms were returned to the field and placed vertically 0–90 cm below the surface of the soil with 10 cm protruding above the soil surface. The microcosms were placed in the holes remaining from the initial soil collection and the sides were packed with soil to minimize atmospheric interaction.

The experimental design was a split plot randomized complete block design with four replicate blocks. Within each block, harvest time (7, 13, or 19 months) was randomly assigned to one of three rows and litter types (root, leaf, or no residue control) were randomly assigned within rows. Three destructive harvests of 12 microcosms (4 replicate × 3 litter types) occurred on June 2nd 2014, December 1st 2014, and June 1st 2015.

2.5 | Litter and bulk soil processing

At harvest, microcosms were removed from the field and placed in a 4°C refrigerator until they were deconstructed the following day. They were cut into three pieces by depth (0–30, 30–60, and 60–90 cm) with a circular saw. Each piece was removed from the PVC placed intact into a sealed plastic bag, and returned to the 4°C refrigerator until being processed within 2 weeks from harvest. All processing and analyses happened individually per tube and depth layer.

Harvested soils were removed from the mesh sock, gently broken to pass through an 8 mm sieve. Roots or leaves greater than 8 mm in length were removed with tweezers and saved as litter mass remaining, while the soil was homogenized and weighed at field moisture. Bulk soil subsamples of ~20 g were dried at 105°C for % moisture and BD determination. The remaining soil was air dried for 3–4 days. The recovered litter was rinsed with deionized water over a 250 μm sieve to remove soil particles and then oven dried at 65°C. Oven-dry soil and leaf or root litter were finely ground and analyzed for %C, %N, δ13C, and δ15N on the EA-IRMS.

2.6 | Soil organic matter fractionation

Macro- and micro-aggregates and free MAOM were isolated by size, and aggregates further fractionated, in order to separate occluded microaggregates and primary SOM pools within aggregates: light fraction (LF), heavy POM, and MAOM.

First, a 75 g bulk soil subsample was wet sieved to separate into three aggregate size classes, macroaggregates (M: >250 μm), free microaggregates (m: 250–53 μm), and free silt and clay-sized organic matter (MAOM: <53 μm), modified from Six, Paustian, Elliott, and Combrink (2000). We did not isolate large macroaggregates (>2,000 μm) as preliminary testing showed they comprised less than 5% of the total soil, likely due to the history of tillage at the site. All fractions were oven dried at 65°C, subsamples of M and m were set aside for further fractionation, and the remainder was finely ground and stored at room temperature in glass jars.

Subsamples of 20 g of the M fraction were dispersed and separated into coarse POM (>250 μm), occluded macroaggregate (Mm: 53–250 μm), and macroaggregate occluded MAOM (M_MAOM: <53 μm), using a microaggregate isolator as described in Six, Elliott, et al. (2000) and Del Galdo, Six, Peressotti, and Cotrufo (2003). All fractions were oven dried at 65°C, finely ground and stored in glass jars at room temperature. Subsamples of Mm were set aside for further fractionation.

Subsamples of m and Mm, were further fractionated by density and size as described in Soong and Cotrufo (2015) into a light fraction (mLF or MmLF: <1.85 g/cm3) and a heavy fraction. The heavy fraction was separated by size into microaggregate (m and Mm) occluded heavy POM (>53 μm) and microaggregate (m and Mm) occluded MAOM (<53 μm). POM and MAOM fractions were oven dried at 65°C, finely ground, and stored in glass jars. LF fractions were too small to be analyzed individually, thus four unground replicates were composited for further analysis. Subsamples from all fractions were analyzed for %C, %N, δ13C, and δ15N on the EA-IRMS. A full characterization in terms of C and N distribution across the different physical fractions of the soil from the control microcosms is provided in Table 1.

2.7 | Data analysis

Litter-C and N contribution to the bulk soil, recovered litter residue, and SOM fractions were assessed for the litter-added plots as compared to the control (no litter-added) plots, using the isotopic mixing model as follows:

\[ f_{\text{litter}} = \frac{\delta_{\text{litter}} - \delta_c}{\delta_{\text{litter}} - \delta_e} \]
where \( f_{\text{litter}} \) is the litter derived C or N fraction of bulk soil, recovered litter, or SOM fraction, \( \delta_s \) is the \( \delta^{13}\text{C} \) or \( \delta^{15}\text{N} \) of the specific C or N pool (bulk soil, recovered litter or SOM fractions), \( \delta_c \) is the \( \delta^{13}\text{C} \) or \( \delta^{15}\text{N} \) of the corresponding control pool, averaged by block across harvests (Table 1), and \( \delta_{\text{litter}} \) is the \( \delta^{13}\text{C} \) or \( \delta^{15}\text{N} \) of the initial litter. The percentage of litter-derived C (LDC) and N (LDN) in these pools were obtained by multiplying the \( f_{\text{litter}} \) values to corresponding C or N pool size of the fraction, then dividing by the amount of litter C or N added to the soil. The LDC and LDN pool in the SOM fractions was calculated for individual samples. Percentage LDC and LDN are used to report the results of this study rather than mg-C or N g-soil in order to be able to directly compare the two litter types with differing initial %C and %N (Table 2).

R (version 3.0.2) was used for statistical analysis, including specific packages lme4, lmerTest, and lsmeans. Separate linear mixed models (fit by REML t tests and Satterthwaite approximations to degrees of freedom) were considered for the following C and N pools: bulk soil, recovered litter, primary and secondary SOM fractions, and total SOM, POM, and MAOM recovered (sum of all three depths). Response variables were % LDC and LDN in each pool and % fraction in total soil. Separate models were fit for each fraction, due to the different scales of response variables across fractions. The model included litter type, sampling time and their interactions as fixed effects and block and block × sampling time as random effects. We checked for normality and homogeneity of variances of the residuals and applied a log-transformation when necessary.

3 | RESULTS

3.1 | Litter chemistry

*Sorghum bicolor* root and leaves had significantly different initial litter chemistry (Table 2), with the leaves characterized, as expected, by a lower biochemical recalcitrance, as shown by the significantly higher HWE (by 62%), and lower C:N (by 29%), and LCI (by 69%) than root litter. Both litters showed relatively low biochemical recalcitrance with the AUR fraction being <10% in both litter types.

3.2 | Litter decay and recovery of litter derived C and N along the soil profile

We measured litter decay by applying the isotopic mixing model to all litter collected from the microcosms at each harvest. This litter also contained new root ingrowth, but isotopic labeling allowed us to accurately trace the fate of the remaining isotopically enriched leaf or root treatment. Our isotopically enriched *S. bicolor* leaf and root litter lost over 99.99% of initial C content and 99.99% of initial N content,
by 19 months of incubation (Figure 1). Exponential decay was observed for C and N loss dynamics from both litter types. Leaf litter C and N had faster rates of loss than root litter C and N:

- Leaf C = 100.00e\(^{-0.0117x}\), \(r^2 = 0.9370\)
- Leaf N = 100.00e\(^{-0.131x}\), \(r^2 = 0.9855\)
- Root C = 100.00e\(^{-0.0113x}\), \(r^2 = 0.8112\)
- Root N = 100.00e\(^{-0.0121x}\), \(r^2 = 0.9107\)

By 19 months of incubation, 19.27% LDC from leaf litter and 26.68% LDC from root litter was recovered in total SOM (\(p = 0.0109\)); 42.48% LDN from leaf litter and 40.21% LDN from root litter was recovered in total SOM. These LDC and LDN recoveries are cumulative across the incubation depth (0–30 cm) and the deeper soil (30–60 and 60–90 cm) for both litter types (Figure 1).

Most of the LDC and LDN were recovered in the incubation depth, though more N moved to the deeper depths than C (Figure 1). Litter type had a main effect on LDC in total SOM (sum of all three depths), in which leaves contributed significantly less to bulk SOM than roots (Table 3, Figure 1a,b). There was no main effect of time and no significant interaction between time and litter type for LDC recovered in bulk SOM; there was no main effect of time or litter type and no interactive effect on LDN in bulk SOM (Table 3).

### 3.3 | Recovery of litter derived C and N in free and aggregate occluded SOM fractions

After initial fractionation of the 0–30 cm bulk soil into M, free m and free MAOM, most of the LDC and LDN was recovered in M, macroaggregate structures (Figure 2). After only 7 months of incubation, 7.65% LDC and 13.91% LDN were recovered from leaf litter and 11.25% LDC and 14.20% LDN were recovered from root litter in the M fraction. Litter type had a main effect on LDC but not on LDN recovered in M. Time had a main effect on LDC and LDN recovered in M, but there was no interaction between the two for LDC or LDN recovered in the M fraction (Table 3). LDC recovered in the M fraction from leaf litter was 32.69% lower than from root litter.
Table 3  Results of the linear mixed model of the effect of sampling time (T), and litter type (L) and their interaction on the % litter derived C and % litter derived N on each soil and SOM fraction (df: degrees of freedom [numerator, denominator]). See Table 1 for explanation of SOM fraction abbreviation.

| % Litter derived C | Effect | df   | F    | p     | % Litter derived N | Effect | df   | F    | p     |
|--------------------|--------|------|------|-------|--------------------|--------|------|------|-------|
| Remaining in litter|        |      |      |       |                    |        |      |      |       |
| T                  | 2, 5.82| 1004.36 | <0.0001 |       | T                  | 2, 5.82| 345.02 | <0.0001 |       |
| L                  | 1, 8.70| 158.73 | <0.0001 |       | L                  | 1, 8.70| 71.58  | <0.0001 |       |
| T × L              | 2, 8.67| 151.68 | <0.0001 |       | T × L              | 2, 8.67| 55.76  | <0.0001 |       |
| SOM 0–30 cm        |        |      |      |       |                    |        |      |      |       |
| T                  | 2, 6   | 0.19 | 0.8307 |       | T                  | 2, 6   | 0.90  | 0.4553 |       |
| L                  | 1, 9   | 25.92| 0.0007 |       | L                  | 1, 9   | 6.14  | 0.0351 |       |
| T × L              | 2, 9   | 2.18 | 0.1689 |       | T × L              | 2, 9   | 4.57  | 0.0428 |       |
| SOM 30–60 cm       |        |      |      |       |                    |        |      |      |       |
| T                  | 2, 6   | 1.00 | 1.4213 |       | T                  | 2, 6   | 2.24  | 0.1873 |       |
| L                  | 1, 9   | 1.24 | 0.2942 |       | L                  | 1, 9   | 3.27  | 0.1041 |       |
| T × L              | 2, 9   | 3.30 | 0.0843 |       | T × L              | 2, 9   | 2.38  | 0.1482 |       |
| SOM 60–90 cm       |        |      |      |       |                    |        |      |      |       |
| T                  | 2, 6   | 1.05 | 0.4067 |       | T                  | 2, 6   | 3.20  | 0.1135 |       |
| L                  | 1, 9   | 1.03 | 0.3362 |       | L                  | 1, 9   | 2.15  | 0.1763 |       |
| T × L              | 2, 9   | 1.13 | 0.3636 |       | T × L              | 2, 9   | 0.38  | 0.6929 |       |
| Total SOM          |        |      |      |       |                    |        |      |      |       |
| T                  | 2, 6   | 0.54 | 0.6069 |       | T                  | 2, 6   | 1.47  | 0.3016 |       |
| L                  | 1, 9   | 46.65| <0.0001 |       | L                  | 1, 9   | 3.31  | 0.1022 |       |
| T × L              | 2, 9   | 0.42 | 0.6695 |       | T × L              | 2, 9   | 3.00  | 0.1005 |       |
| M                  |        |      |      |       |                    |        |      |      |       |
| T                  | 2, 6   | 8.97 | 0.0158 |       | T                  | 2, 6   | 14.55 | 0.0050 |       |
| L                  | 1, 9   | 14.75| 0.0040 |       | L                  | 1, 9   | 0.71  | 0.4202 |       |
| T × L              | 2, 9   | 0.06 | 0.9431 |       | T × L              | 2, 9   | 0.87  | 0.4489 |       |
| Free m             |        |      |      |       |                    |        |      |      |       |
| T                  | 2, 6   | 0.39 | 0.6918 |       | T                  | 2, 6   | 0.30  | 0.7481 |       |
| L                  | 1, 9   | 2.54 | 0.1453 |       | L                  | 1, 9   | 1.03  | 0.3358 |       |
| T × L              | 2, 9   | 3.97 | 0.0580 |       | T × L              | 2, 9   | 4.69  | 0.0402 |       |
| Free MAOM          |        |      |      |       |                    |        |      |      |       |
| T                  | 2, 6   | 2.11 | 0.2021 |       | T                  | 2, 6   | 0.06  | 0.9422 |       |
| L                  | 1, 9   | 0.00 | 0.9991 |       | L                  | 1, 9   | 1.14  | 0.3128 |       |
| T × L              | 2, 9   | 1.55 | 0.2641 |       | T × L              | 2, 9   | 1.51  | 0.2721 |       |
| Total LF           |        |      |      |       |                    |        |      |      |       |
| T                  | 2, 6   | 27.33| 0.0010 |       | T                  | 2, 6   | 5.54  | 0.0434 |       |
| L                  | 1, 9   | 18.09| 0.0021 |       | L                  | 1, 9   | 4.50  | 0.0629 |       |
| T × L              | 2, 9   | 12.99| 0.0022 |       | T × L              | 2, 9   | 12.79 | 0.0023 |       |
| Total POM          |        |      |      |       |                    |        |      |      |       |
| T                  | 2, 6   | 20.09| 0.0022 |       | T                  | 2, 6   | 22.86 | 0.0016 |       |
| L                  | 1, 9   | 11.43| 0.0081 |       | L                  | 1, 9   | 1.08  | 0.3261 |       |
| T × L              | 2, 9   | 7.00 | 0.0146 |       | T × L              | 2, 9   | 5.26  | 0.0306 |       |
| Total MAOM         |        |      |      |       |                    |        |      |      |       |

(Continues)
litter \((p = 0.0370)\) at 13 months of incubation. While persisting, this difference was no longer significant \((p = 0.0516)\) after 19 months of incubation. Within the leaf litter treatment, the LDC and LDN recovered in M increased over time by 67.56\% \((p = 0.0461)\) and 82.42\% \((p = 0.0054)\), respectively. Within the root litter treatment, the LDC and LDN recovered in M increased over time by 47.91\% \((p = 0.0270)\) and 74.63\% \((p = 0.0095)\), respectively (Figure 2). There was no main effect of litter type or time for LDC or LDN recovery in free m or free MAOM. There was an interaction between time and litter type for LDN recovery in m (Table 3).

Aggregates (M, Mm, and m) were further separated into LF, heavy POM and MAOM. For the M fraction, LF and heavy POM are not separated but maintained within one fraction which we refer to as “Coarse POM” consistently with previous studies (Del Galdo et al., 2003; Six, Elliott, et al., 2000). Main effects

| Effect | df | F    | p    | Effect | df | F    | p    |
|--------|----|------|------|--------|----|------|------|
| T      | 2, 6 | 16.69 | 0.0035 | T      | 2, 6 | 14.09 | 0.0054 |
| L      | 1, 9 | 1.06  | 0.0262 | L      | 1, 9 | 2.62  | 0.1402 |
| T × L  | 2, 9 | 0.24  | 0.7899 | T × L  | 2, 9 | 1.48  | 0.2772 |

**Coarse POM**

| Effect | df | F    | p    | Effect | df | F    | p    |
|--------|----|------|------|--------|----|------|------|
| T      | 2, 5.87 | 0.41  | 0.6843 | T      | 2, 5.83 | 0.61  | 0.5751 |
| L      | 1, 8.55 | 45.57 | 0.0001 | L      | 1, 8.57 | 40.30 | 0.0002 |
| T × L  | 2, 8.51 | 2.14  | 0.1770 | T × L  | 2, 8.54 | 1.32  | 0.3157 |

**m LF**

| Effect | df | F    | p    | Effect | df | F    | p    |
|--------|----|------|------|--------|----|------|------|
| T      | 2, 5.79 | 8.67  | 0.0181 | T      | 2, 5.79 | 9.96  | 0.0133 |
| L      | 1, 8.61 | 0.03  | 0.8620 | L      | 1, 8.65 | 10.60 | 0.0104 |
| T × L  | 2, 8.58 | 13.23 | 0.0024 | T × L  | 2, 8.62 | 11.26 | 0.0039 |

**Mm LF**

| Effect | df | F    | p    | Effect | df | F    | p    |
|--------|----|------|------|--------|----|------|------|
| T      | 2, 6 | 57.43 | 0.0001 | T      | 2, 6 | 24.87 | 0.0012 |
| L      | 1, 9 | 50.79 | <0.0001 | L      | 1, 9 | 1.5  | 0.7071 |
| T × L  | 2, 9 | 7.75  | 0.0110 | T × L  | 2, 9 | 4.32  | 0.0483 |

**m Heavy POM**

| Effect | df | F    | p    | Effect | df | F    | p    |
|--------|----|------|------|--------|----|------|------|
| T      | 2, 5.92 | 0.52  | 0.6170 | T      | 2, 5.92 | 0.53  | 0.6138 |
| L      | 1, 8.47 | 3.36  | 0.1019 | L      | 1, 8.48 | 1.88  | 0.2053 |
| T × L  | 2, 8.44 | 1.59  | 0.2592 | T × L  | 2, 8.45 | 2.40  | 0.1496 |

**Mm Heavy POM**

| Effect | df | F    | p    | Effect | df | F    | p    |
|--------|----|------|------|--------|----|------|------|
| T      | 2, 6 | 47.04 | 0.0002 | T      | 2, 6 | 30.44 | 0.0007 |
| L      | 1, 9 | 11.24 | 0.0085 | L      | 1, 9 | 0.45  | 0.5170 |
| T × L  | 2, 9 | 5.97  | 0.0224 | T × L  | 2, 9 | 3.50  | 0.0750 |

**M MAOM**

| Effect | df | F    | p    | Effect | df | F    | p    |
|--------|----|------|------|--------|----|------|------|
| T      | 2, 6 | 4.96  | 0.0535 | T      | 2, 6 | 9.12  | 0.0152 |
| L      | 1, 9 | 4.08  | 0.0741 | L      | 1, 9 | 0.46  | 0.5161 |
| T × L  | 2, 9 | 0.79  | 0.4831 | T × L  | 2, 9 | 1.36  | 0.3046 |

**m MAOM**

| Effect | df | F    | p    | Effect | df | F    | p    |
|--------|----|------|------|--------|----|------|------|
| T      | 2, 5.96 | 0.64  | 0.5604 | T      | 2, 5.86 | 0.54  | 0.6075 |
| L      | 1, 8.32 | 0.21  | 0.6549 | L      | 1, 8.62 | 1.30  | 0.2842 |
| T × L  | 2, 8.30 | 2.38  | 0.1528 | T × L  | 2, 8.59 | 1.02  | 0.3993 |

**Mm MAOM**

| Effect | df | F    | p    | Effect | df | F    | p    |
|--------|----|------|------|--------|----|------|------|
| T      | 2, 6 | 32.72 | 0.0006 | T      | 2, 6 | 24.15 | 0.0014 |
| L      | 1, 9 | 12.71 | 0.0061 | L      | 1, 9 | 9.76  | 0.0122 |
| T × L  | 2, 9 | 0.57  | 0.5853 | T × L  | 2, 9 | 1.73  | 0.2317 |
of litter type and time varied by primary fraction. Most of the activity was found in the microaggregates occluded within macroaggregates (Mm) (Figure 3), with most of the LDC and LDN recovered for each litter type in the occluded MAOM fractions (Figure 3). Less leaf LDC was recovered in POM and LF fractions than root, particularly within occluded microaggregates (Figure 3). Litter type had a main effect on LDC and LDN recovered in Coarse POM, but time did not (Table 3). There was a main effect of litter type and time and an interaction between the two for LDC and LDN recovered in total LF fractions (sum of m_LF and Mm_LF) (Table 3). While the LF pools varied over time within each treatment, by 19 months of incubation LDC and LDN recovered from leaf litter was not significantly different than those recovered from root litter for total LF (p = 0.6606 and p = 0.0888, respectively). In total LF, LDC, and LDN from leaf litter tended to decrease over time, with significantly less recovered from 7 to 19 months of incubation (p = 0.0042 and p = 0.0160). However, LDC and LDN recovered in total LF from root litter increased initially from 7 to 13 months of incubation (p = 0.0017 and 0.0062), and then decreased again from 13 to 19 months of incubation (p < 0.0001 and p = 0.0016).

There was a main effect of litter type and time on LDC recovered in total POM (sum of all POM fractions in m and Mm), and a main effect of time but not litter type on LDN recovered in total POM (Table 3). There was an interaction between litter type and time for LDC and LDN recovered in total POM. At 19 months of incubation, total POM LDC recovered from leaf litter was 35% less than LDC recovered from root litter (p = 0.0364). At 13 months of incubation total POM LDN recovered from leaf litter was 47% less than that from root litter (p = 0.0180), but was no longer significantly different at 19 months of incubation (p = 0.5549).

There was a main effect of litter type on LDC recovered in total MAOM (sum of free MAOM, m_MAOM, Mm_MAOM and M_MAOM), but not LDN (Table 3). In total MAOM, LDC recovery was 21.91% and LDN recovery was 14.88% greater from leaf litter than from root litter (Figure 3). There was a main effect of time on LDC and LDN recovered in total MAOM (Table 3). When looking more closely at the free and occluded MAOM fractions, there was no interaction between litter type and time on any individual MAOM

**FIGURE 2** *Sorghum bicolor* leaf- and root-derived (a) Carbon and (b) Nitrogen recovered in the macroaggregate (M), microaggregate (m) and free mineral-associate organic matter (MAOM) fractions within the depth layer where the litter was incubated (0–30 cm), for the three time harvests during the 19 months of field incubation. Amounts are given in % of the initial litter residue at time 0. Data are average with standard errors (n = 4)
fraction. However, litter type and time had main effects on LDC and LDN in Mm_MAOM (Table 3).

4 | DISCUSSION

Removal of aboveground biomass from *S. bicolor* for biofuel production requires understanding differential contribution of leaf and root residue inputs to soil C and N storage and recycling, and to identify potential impacts that this removal could have on the soil. In this study we demonstrated that the chemical composition of leaf and root *S. bicolor* residues affect their contribution to SOM C and N pools along the soil profile and across different SOM physical fractions. This is consistent with the most recent frameworks suggesting higher MAOM formation from labile residues and higher POM formation from recalcitrant residues (Castellano et al., 2015; Cotrufo et al., 2013). Furthermore we highlighted the important role of aggregates for the efficient formation of MAOM in a highly structured soil.

Our in situ litter incubation experiment confirms that roots and leaves from *S. bicolor* significantly differ in their inherent chemical composition and recalcitrance to decomposition. This resulted in different contribution to C and N in bulk soil, as well as different allocation of the newly formed SOM among physical fractions characterized by different mechanisms of protection. Despite being grown in a labeling chamber (Soong et al., 2014), the chemistry of our litters were similar to others reported for sorghum, with the exception of somewhat low %AUR (a lignin and suberin proxy) (Stewart, Moturi, Follett, & Halvorson, 2015; Zhao et al., 2009). In every measure of litter decomposability (i.e., %HWE, C:N and LCI), sorghum leaves were of lower biochemical recalcitrance than roots (Table 2). However, both litter types had relatively low %AUR, and therefore overall were fairly decomposable. This was demonstrated by the rapid mass loss of both residues, which reached complete decomposition after only 19 months of incubation. In agreement with its relatively higher lability, the leaf litter decomposed faster than the root litter (Figure 1). Similar to other experiments conducted without confining the litter in mesh bags (e.g., Soong et al., 2016), this experiment confirmed that when left free to decompose in the soil plant residue reaches complete mass loss in a few years; subsequently, a significant fraction of the original mass is found in different forms of SOM. Moreover, the relatively fast decomposition observed in this study could have been facilitated by incubation of the litter within the top soil in close proximity to the soil matrix, where plant litter
has been shown to decompose faster and produce SOM more efficiently than when incubated on the soil surface (Mitchell et al., 2016, 2018).

The different litter chemistry between our sorghum roots and leaves not only affected the different decay rates of the two litter types, but also the efficiency with which litter C (but surprisingly not N) was retained in bulk soil across the entire soil profile. We found that after 19 months of incubation 27% of the initial root C was retained in the bulk soil overall, vs 19% of the leaf litter C. This suggests that in crops where roots are inherently more recalcitrant than above ground biomass, if root production is increased this can potentially offset some of the losses from above ground input removal. This offset could be further increased by the fact that root inputs are released within the soil, at least in a no till system, while leaf litter is deposited on the soil surface where SOM formation is deemed to be less efficient (Mitchell et al., 2016, 2018; Sokol et al., 2019). Most of the LDC and LDN were found in the layer where litter was incubated, with little vertical movement (Figure 1), emphasizing the importance of root inputs to depth to accrue SOM in those deeper layer (Rumpel & Kögel-Knabner, 2010). Within the top 0–30 cm, root and leaf litter contributed significantly different amounts not only of LDC but also of LDN to new SOM. In fact, an average of 35% of the root LDN was retained in bulk soil in the 0–30 cm vs an average of 28% from the leaves. This difference might have been due to the higher overall C retention from root litter, as well as the higher root C:N (Table 1) which potentially stimulated N immobilization in root litter-derived SOM to meet the stoichiometric needs of the microbes (Manzoni, Jackson, Trofymow, & Porporato, 2008). Plant inputs not only provide C for SOM accrual, but through their mineralization they also recycle N to sustain productivity (Janzen, 2006). Clearly while increasing more recalcitrant root inputs can be an effective means to increase SOM accrual, it seems less effective at providing fertility through N mineralization, with lower overall inputs due to lower %N (Table 1) and higher relative retention of N in soil. It is noteworthy that our experiment was short-term, and that more root LDN could be made available over time from the mineralization of the POM fractions (Austin, Wickings, McDaniel, Robertson, & Grandy, 2017), which were higher in the root litter treatment (Figure 3). If above ground biomass is removed in bioenergy crops, a balanced alternative addition of organic C and N inputs needs to be implemented to sustain crop production at the same time of SOM accrual.

Examining only the bulk soil does not adequately capture SOM formation dynamics, nor allow predicting the persistence of the newly formed SOM. Aggregates are universally recognized as important SOM structures which offer physical protection to SOM from decomposition in the short-term (Jastrow, 1996). Depending on the system, macroaggregates are expected to turnover within years to decades and microaggregates within decades to centuries (Lützow et al., 2007). However, the potentially strong role that aggregates play in new SOM formation is less well understood. We demonstrated that in a highly aggregated soil, SOM formation from the decomposition of plant debris starts within aggregates. After only 7 months of litter incubation, the large majority of the LDC and LDN were found in macro- and micro-aggregate structures, increasing over time, while the LDC and LDN in free MAOM was only a minor fraction and did not significantly differ over time (Figure 2, Table 3). This finding supports the hypothesis that input of plant debris promotes aggregate formation (Six, Bossuyt, Degryze, & Denef, 2004), and simultaneously emphasizes the dynamic, active nature of aggregates as a place where SOM, and particularly MAOM, is formed and transformed (Jastrow, 1996; Lehmann, Kinyangi, & Solomon, 2007) over the generalized idea of aggregates as a place where decomposition is inhibited through spatial inaccessibility (Lützow et al., 2006).

While aggregates are important SOM structures they are composite pools and examining SOM formation only at their level prevents from identifying underlying pathways. When aggregates are disrupted and the primary SOM fractions (i.e., LF, POM, MAOM) within analyzed, then the actual SOM dynamics can be effectively studied (Del Galdo et al., 2003; Six, Elliott, et al., 2000). In our study, the contribution to primary SOM fractions differed significantly between litter types: we found overall greater contribution to MAOM from the more labile leaf litter and greater contribution to particulate fractions (i.e., coarse POM, LF and heavy POM) from the more recalcitrant root litter. This observation confirmed the hypothesis that labile litter forms MAOM efficiently in soils with high matrix capacity, consistently with the Microbial Efficiency Mineral Stabilization framework (Cotrufo et al., 2013), while recalcitrant litter results in more POM formation (Castellano et al., 2015). It is also consistent with the few studies that compared SOM formation from root and shoot residues. Contribution to SOM formation from roots is often found to be much higher than from leaves (Berhongaray, Cotrufo, Janssens, & Ceulemans, 2018; Sokol et al., 2018), but leads to higher POM or POM like fractions (Bird et al., 2008), while leaf litters often exhibit a relatively higher contribution to MAOM or persistent SOM fractions (Hatton, Castanha, Torn, & Bird, 2015; Lalalve et al., 2018). While supporting the idea that more SOM can be formed from root residues in annual crops where they often have a higher recalcitrance than above-ground residues, these findings suggest that the mean residence time of root residues-derived SOM is not higher than that formed from above-ground residue decomposition. This conflicts with Rasse, Rumpel, and Dignac (2005), who found that the mean residence time of root-derived C in soils is 2.4 times greater than shoot-derived C, and Jackson et al. (2017) who suggested root inputs are five times
as likely to be stabilized as SOM, perhaps higher in agricultural systems. Indeed, this study only investigated residues inputs, and root exudates given their inherent lability may efficiently form persistent SOM (Cotrufo et al., 2013; Sokol et al., 2018; Strickland, Wickings, & Bradford, 2012). We believe that the fractionation of SOM into physical fractions of known stabilization mechanisms can help predict the persistence of residue derived C and N in soil.

The higher MAOM formation from the leaf litter characterized by high HWE, and the higher POM formation from the root litter characterized by a higher LCI generally support the two pathways model of SOM formation (Cotrufo et al., 2015): dissolved organic matter from non-structural compounds (corresponding to high HWE-C) is available early in decomposition contributing efficiently to MAOM directly and via microbial processing (Liang et al., 2017), while POM forms through the fragmentation and partial transformation of recalcitrant structural litter components (corresponding to high HWE-C) is available early in decomposition contributing efficiently to MAOM directly and via microbial processing (Liang et al., 2017). However, our complete fractionation scheme and repeated sampling over time allowed identifying more subtle dynamics. First, it is important to notice that what we define as particulate structures include both little transformed plant derived light materials (LF) and heavy POM, more microbially processed and denser organic matter coating sand (Christensen, 2001). In our fractionation we have the two together inside macro-aggregates as Coarse POM, but they were separated within microaggregates in LF and heavy POM (Figure 3). By following the LDC in these fractions over time we can identify more specific SOM formation and transformation processes. Independent of litter types, inside micro-aggregate the decomposition of litter structural components first formed LF and then heavy POM, likely as a result of LF decomposition (Figure 3). This dynamic is in agreement with the general understanding of heavy sand-sized POM resulting from the microbial processing of plant derived LF (Grandy & Neff, 2008). Differently from this cascade model and in agreement with the two-pathways model, in our study MAOM formation appeared to be independent of LF and heavy POM dynamics, and formed during the active phases of litter mass loss (Figures 1 and 2). In addition to the HWE, non-lignin encrusted structural materials were abundant in both litter and must have been active contributors to the formation of MAOM. Particularly, we observed a continued increase in MAOM formation in the period between 7 and 13 months, when these structures are expected to degrade (McKee, Soong, Calderon, Borch, & Cotrufo, 2016); MAOM remained largely stable thereafter (Figure 3).

Overall, MAOM is the SOM fraction deemed to be the most persistent and resistant to disturbance (Lützow et al., 2006), and thus the fraction targeted for long-term C sequestration. Although, it is important to note that some of the new MAOM formation we observed may be the result of surface exchanges rather than net accrual (Jilling et al., 2018), S. bicolor root and leaf residue inputs were relatively labile and had MAOM formation efficiencies of 7.4% and 9.1%, respectively. Thus, both represent a good source of persistent SOM, in particular in clay-loam highly aggregated soils. Carbon sequestration in MAOM, however, has a high N cost. While MAOM is now looked at as a significant reservoir of N (Jilling et al., 2018), mobilizing N from MOAM to sustain fertility will reduce persistent C storage, opening the well-known “soil C dilemma” (Janzen, 2006). Soil fertility must be assured without jeopardizing SOM resources through the input of N rich residues, for example through legume cover crops (Schipanski et al., 2014). These issues emphasize the importance of considering the quantity of root or shoot inputs in order to meet the competing needs for SOM pools when managing agricultural production systems.

From both litter types, we demonstrated rapid formation of MAOM within macroaggregates, thought to be an important step in the process of microaggregate formation within macroaggregates (Jastrow, 1996; Oades, 1984; Six et al., 2004). We also saw continued increases in occluded microaggregate MAOM over time (Figure 3), further emphasizing the dynamic nature of aggregates and their key role in the formation of persistent organo-mineral bonding, rather than in the physical inhibition of SOM decomposition through occlusion (Lehmann et al., 2007). If increasing root inputs is pursued as a strategy to sequester C during bioenergy feedstock production, the nature and long-term persistence of the SOM accrued needs to be assessed. From this study, root residues appeared effective at forming new SOM, but relatively more in unstable particulate fractions. While stalks would constitute the majority of aboveground residue in sorghum production after grain is harvested we used leaves in this study, since both leaves and stalks were similar in their litter chemistry and more labile than roots. Stalks had the highest %HWE-mass of the three litter types given their high sugar content, so it is likely that they would have contributed even more efficiently to MAOM production than leaves. Thus, our study may have underestimated the ability of sorghum residues to contribute to this more stable SOM. However, if the aboveground materials are harvested for lignocellulosic ethanol production most of the stalks would be removed.

We also demonstrated that aggregates are a place of efficient residue decomposition and MAOM formation. The promotion of aggregation in the mineral soil through live and dead root inputs (Denef & Six, 2006) may thus be an important factor stimulating persistent SOM production in root enhanced bioenergy crops.

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