Roots selectively decompose litter to acquire nitrogen and build new soil carbon

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Abstract

Plant-microbe interactions in the rhizosphere shape carbon and nitrogen cycling in soil organic matter (SOM). However, there is conflicting evidence on whether these interactions lead to a net loss or increase of SOM. In part, this conflict is driven by uncertainty in how living roots and microbes alter SOM formation or loss in the field. To address these uncertainties, we traced the fate of isotopically labeled litter into SOM using root and fungal ingrowth cores incubated in a Miscanthus x giganteus field . Roots stimulated litter decomposition, but balanced this loss by transferring carbon into more persistent, aggregate associated SOM. Further, roots selectively mobilized nitrogen from litter without additional carbon release. Overall, our fundings suggest that roots can efficiently mine nitrogen and build persistent soil carbon.

Introduction:

Managing soils in agricultural systems to sequester carbon (C) in soil organic matter (SOM) may be a powerful approach to offset anthropogenic C emissions (Lal, 2004). Soils are the largest terrestrial C pool, and experimental manipulations like changing vegetation type, increasing organic inputs, or altering management practices demonstrate the potential for significant and rapid SOM accumulation (Minasny et al., 2017; Paustian et al., 2016). However, there is a high degree of uncertainty in understanding, predicting, and optimizing soil C accumulation (Sulman et al., 2018). Much of this uncertainty arises because plant roots and soil microbes, the active drivers of soil biogeochemical cycling, both build and deplete SOM through simultaneously occurring processes. As such, our ability to optimize soil C sequestration relies on improving our understanding of how roots and microbes drive the transfer of new litter C inputs into SOM.

As per the current understanding of SOM formation, litter inputs are decomposed into simpler compounds that can be physically protected from microbial decomposers by occlusion in soil aggregates or sorption to mineral surfaces (Lehmann & Kleber, 2015). As such, SOM is often delineated into three main pools (Fig. 1a): undecomposed or partially-decomposed particulate organic matter (here, light POM), aggregate-occluded SOM (here, heavy POM), and mineral associated organic matter (MAOM) (Lavallee et al., 2020). Light POM accumulation depends upon the balance between litter inputs to soil and litter decomposition, and can accumulate with no apparent upper limit but is also vulnerable to factors like warming that enhance decomposition rates (Benbi et al., 2014; Cotrufo et al., 2019). Heavy POM is operationally separated from light POM by density fractionation and is linked with stable soil aggregates (Lavallee et al., 2020). Accumulation in this pool may saturate and is vulnerable to factors like soil disturbance and land use change (Bronick & Lal, 2005). MAOM is generally considered to be the most persistent or protected form of SOM (Cotrufo et al., 2013; Liang et al., 2017). However, optimizing MAOM accumulation may only be practical in soils like those in degraded agricultural ecosystems that have lost nearly 50% of their C since ploughing the prairie (Stockmann et al., 2015) as MAOM accumulation appears to saturate (Cotrufo et al., 2019; but see Georgiou et al., 2022). To manage ecosystems for soil C sequestration, it is critical to

understand what drives the transfer of new litter inputs between these SOM pools to enhance our predictive understanding of how much soil C can accumulate and how persistent this soil C may be in a changing climate.

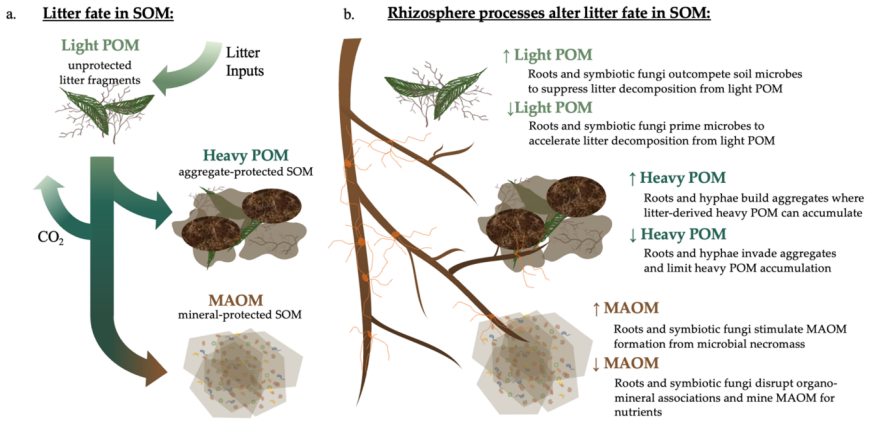

Figure 1a: Litter inputs join SOM as light POM, which is largely composed of undecomposed litter fragments. As decomposition progresses, litter-derived SOM can more easily become incorporated into aggregates in heavy POM or microbial decomposition products and necromass can preferentially sorb to soil mineral surfaces as MAOM.**1b:** Roots and root-associated fungal symbionts can enhance both retention or loss of litter in light POM (top), heavy POM (middle), and MAOM (bottom) pools.

Living roots and their associated fungi alter SOM formation by sending C-rich exudates to the rhizosphere to enhance decomposition and acquire N (Bais et al., 2006; Grayston et al., 1997). However, a high degree of uncertainty remains in whether this increases or decreases soil C accumulation. In Figure 1b, we diagram potential hypotheses for how roots could alter litter loss from light POM and the accumulation of new heavy POM and MAOM through distinct mechanisms. First, root stimulation of microbial decomposition to mineralize soil N can increase the loss of unprotected light POM through the rhizosphere priming effect (Cheng et al., 2014). However, there is also evidence that roots and symbiotic fungi can outcompete saprotrophic microbes for resources like water and nutrients leading to the suppression of decomposition (Fernandez & Kennedy, 2016). Second, as litter inputs are transferred into more protected heavy POM, root ingrowth has the potential to both invade aggregates and increase the formation rate of new aggregates (Six et al., 2000). Finally, roots can enhance new MAOM formation by increasing the efficiency of microbial litter decomposition, resulting in greater microbial biomass production and the formation of microbial necromass (Liang et al., 2017). This necromass can associate with mineral surfaces and is the main precursor to MAOM in grassland ecosystems (Angst et al., 2021). However, roots may also deplete new, litter-derived MAOM as recent evidence suggests that roots can actively mine MAOM for nutrients (Jilling et al., 2021) and that root exudate compounds can displace MAOM from soil minerals (Keiluweit et al., 2015). As such, predicting whether roots will drive a net gain or loss of soil C is hindered by uncertainty in how roots impact SOM formation in these different pools.

The extent to which roots and mycorrhizal fungi facilitate SOM formation or loss in agricultural ecosystems may be modulated by fertilization. For example, some N-limited plants can dynamically shift C allocation belowground to root exudation and mycorrhizal symbionts to stimulate microbial decomposition in the rhizosphere and increase N acquisition (Brzostek et al., 2014; Kane et al., 2022). When N limitation is alleviated by fertilization, plants can also reduce belowground C allocation, suppressing SOM decomposition (Eastman et al., 2021; Frey et al., 2014). The degree to which fertilization alters SOM cycling also depends upon the activity of saprotrophic soil microbial decomposers. In contrast to plants, soil microbes are primarily understood to be energy, or carbon, limited (Soong et al., 2020). As such, organic fertilizer that contains

C and N can prime microbial activity and decomposition relative to inorganic N fertilizer (Cui et al., 2022; Ndung'u et al., 2021). However, uncertainty remains in the extent to which the priming of microbial activity leads to net soil C losses by enhancing decomposition or net C gains by promoting the production of microbial necromass that can form MAOM. Collectively, the effect of fertilization on SOM formation depends upon the strength of plant-microbe interactions and the form of fertilizer applied, but the magnitude of this effect is uncertain.

Given the uncertainty above, our objectives were to: **1) determine how living roots and symbiotic fungi influence litter decomposition and SOM formation in distinct SOM pools and 2) assess how microbially-driven SOM formation is altered by fertilization.** For the first objective, we assayed the net effect of the opposing hypotheses illustrated in Figure 1. For the second objective, we tested two hypotheses: (1) the effect of living roots on SOM formation would be strongest in unfertilized soil and (2) organic fertilizer would accelerate microbial decomposition and SOM cycling to a greater extent than inorganic fertilizer (SI Figure **1**). To meet our objectives, we measured the effects of living roots and fungi on new SOM formation from isotopically enriched litter over one growing season. We incubated litter inputs in soil cores that were open to roots and fungal ingrowth (root), that excluded roots but were open to fungal ingrowth (fungal), or that excluded both roots and fungi (none) to quantify the effect of living roots and fungi on new SOM formation (SI Fig.**2**). We installed ingrowth cores in *Miscanthus x giganteus* (herein miscanthus) plots with different nutrient treatments to investigate the effect of soil N and C availability on how roots, mycorrhizal fungi, and saprotrophic microbes drive the transfer of litter C and N into light POM, heavy POM, and MAOM. We used the bioenergy feedstock crop miscanthus as a study system because it produces extensive root systems to overcome nutrient limitation (Dohleman & Long, 2009; Heaton et al., 2008) and because miscanthus agriculture typically increases SOM levels (Harris et al., 2015). Further, because bioenergy offers the potential to become a C neutral or C negative alternative to fossil fuels, it is particularly critical to investigate what drives SOM accumulation in these ecosystems (Hanssen et al., 2020).

We show that miscanthus roots increased litter decomposition but did not lead to a net C loss because roots enhanced the incorporation of litter C into heavy POM. Roots also selectively mobilized litter N from both POM pools. As such, roots can transfer C into a more persistent SOM pool while still enhancing N mining. These root effects did not depend on fertilization. However, organic fertilization enhanced microbial litter decomposition without increasing litter incorporation in MAOM.

Materials and methods:

Site description and location selection

This experiment was performed at the West Virginia University (WVU) Animal Sciences farm in Morgantown, West Virginia (39°40'10.2"N, 79°55'53.6"W). This site is located next to the former Baker's Ridge Mine Site (National Mine Repository 304559) and is managed as a cool-season grass pasture (detailed site description available in Kane et al. 2023, *in review*). Miscanthus plots were established in 2019 using a fully randomized block design with 4 fertilization treatments replicated 8 times for a total of 32 plots (Kane et al. 2023,*in review*). Each plot is 5 m^2 and was established by planting 25 miscanthus rhizomes using 1 m^2 grid spacing (site map, SI Fig. **3a**). Plots are fertilized yearly with treatments that include no fertilization, low-level inorganic N additions (28.5 kgN/ha), high-level inorganic N additions (57 kgN/ha), and organic fertilization (local manure, ~57 kgN/ha). Due to logistical constraints for sample size, we utilized the control, high-level inorganic, and organic fertilization treatments for this experiment.

Experimental design

We incubated isotopically enriched litter in soil ingrowth cores and traced the fate of litter C and N into SOM over one growing season. Our experimental design included 3 levels of root/hyphal ingrowth: root and fungal ingrowth (root), root exclusion and fungal ingrowth (fungal), and root and fungal exclusion (none) and 3 fertilization treatments: no fertilization (control), high-level inorganic fertilization (high N), or organic fertilization (organic). We randomly selected 5 plots from each fertilization treatment from those which had successful rhizome establishment during initial plot development. Within each plot, we replicated each

ingrowth core treatment twice, where we installed ingrowth cores by 2 of the plot's 25 plants (SI Fig. **3b**). This resulted in a total of 90 experimental ingrowth cores (3 cores x 3 fertilization treatments x 5 plots x 2 locations/plot).

Ingrowth core construction and installation

Ingrowth core treatments included root and fungal ingrowth (root), root exclusion and fungal ingrowth (fungal), and root and fungal exclusion (none) (SI Fig. **2**). Each ingrowth core was constructed with 10 cm long, 4.5 cm diameter rigid plastic 5 mm mesh tubing. The top 2.5 cm of each core was inserted into 5cm long PVC collars and attached with elastic sealant. Mesh bases were sewn onto each core with 12 lb. nylon fishing line and each core was wrapped with mesh that was glued on with 100% silicon adhesive. Root and fungal ingrowth (root) cores were constructed with 1.5 mm polyacrylic mesh that allowed fine root ingrowth. Root exclusion (fungal and none) cores were constructed with 50 um nylon mesh that was too fine for root ingrowth but allowed hyphal ingrowth (Phillips et al., 2012). Root and fungal exclusion (none) cores were constructed with the same root exclusion mesh and were also twisted once or twice a week to break off hyphae and prevent significant fungal ingrowth and establishment (SI Fig. **2**).

Ingrowth cores were prepared in the lab using isotopically enriched litter amendments and soil harvested from the corresponding plot. In April 2021, soils from the top 10 cm were collected from each future ingrowth core location and were brought back to the lab where they were sieved to 2 mm and stored at 5°C when not being processed. Soils were homogenized within each nutrient treatment (control, high N, or organic) and were mixed with sand that had been acid washed and separated from particles less than 53 um diameter in a 9:1 soil:sand ratio to prevent soil compaction. 250 mg of isotopically enriched corn leaf litter, generated as described in Ridgeway et al., 2022, was used as the substrate in each ingrowth core. This addition rate was selected to be lower than litter production at the site to limit experimental artefacts from introducing a new decomposition substrate and high enough to ensure that the ^{13}C inputs were traceable into SOM pools. This litter had a %C of 41.7% (±0.17%), C:N of 18.8 (±0.64), $\delta^{13}C$ of 7020 (±49), and δ ^{15}N of 34,800 (±310) and was dried and coarsely ground. Each core was filled with corresponding soil, and the labeled litter was gently mixed in to the top 2 cm.

Within 5 days of initial soil collection, the assembled cores were transported to the field location where they were installed into the top 10 cm of soil in each corresponding treatment plot (SI Fig.**3a**). This occurred in April 2021 when miscanthus shoots were beginning to emerge. Within each plot, ingrowth cores were installed 8" north of visibly emerged miscanthus shoots (SI Fig. **3b**). After 20 weeks, the ingrowth cores were carefully cut from the soil in September 2021 and were brought back to the lab for processing. Although each treatment combination began with a planned replicate of n=10, two cores were removed from analysis due to animal interference. Additionally, five cores intended for the root exclusion fungal ingrowth treatment (fungal) were invaded by roots. After determining that these cores did not significantly vary from the rest of the root ingrowth (root) cores, these were also analyzed as root ingrowth (root) cores. Given these adjustments, the total replication ranged from 5-15 for each treatment (provided in SI table 1).

Soil fractionation

Ingrowth cores were destructively harvested in September and litter C and N inputs were traced into SOM pools (Ridgeway et al., 2022). A 5 g subsample of dry soil from each core was separated into light POM, heavy POM, and MAOM by density and size fractionation as described in Lavallee et al. (2020). In brief, the light POM was separated through density floatation in 1.85 g/mL sodium polytungstate salt solution. The remaining soil was separated into heavy POM and MAOM fractions by size separation where the MAOM fraction passes through a 53 um sieve.

Tracing litter C and N fate

To trace the fate of ^{13}C and ^{15}N litter amendments, the soil fractions were analyzed for %C, %N, $\delta^{13}C$, and δ ^{15}N using a Thermo Fisher Delta V+ isotope ratio mass spectrometer interfaced with a Carlo Erba NC2500 Elemental Analyzer. First, the proportion of litter-derived C or N in each soil fraction (f_{litter}) was

determined with two endmember mixing models (eq. 1) (Derrien & Amelung, 2011; Poeplau et al., 2018). Here, the C and N isotope signatures were measured from the enriched litter substrate and each of the 3 SOM fractions from control, high N, and organic soils.

eq. 1: $f_{litter} = \frac{sample\ isotope\ signature - SOM\ isotope\ signature}{litter\ isotope\ signature - SOM\ isotope\ signature}$

Next, the litter C and N recovered in each SOM pool (shown in Fig.**2** , Fig. **5**) was determined for each ingrowth core (eq. 2). Here, the mass proportion of each SOM fraction was determined from lab fractionation and the %C, %N, $\delta\ ^{13}C$, and $\delta^{15}N$ were measured on an elemental analyzer as described above. The distribution of litter C between the SOM fractions (shown in Fig. **3**) was calculated as the litter mass in each SOM fraction out of the total litter mass remaining in the ingrowth core soil.

eq. 2: litter mass = dry soil mass in each core × SOM fraction mass proportion × SOM fraction %C/100 or %N/100 $\times f_{litter}$

Root biomass, root colonization, and microbial biomass

All roots that were inside of the cores were separated from soils and washed in the lab. Dry root biomass was measured, and microbial biomass C was measured from a subsample of soil from each core using chloroform slurry fumigations (Witt et al., 2000) followed by persulfate digestion to CO_2 (Doyle et al., 2004; Kane et al., 2022). In brief, soils were extracted in potassium sulfate with and without chloroform for 4 hrs. Filtered supernatant was digested in persulfate solution where dissolved C was oxidized to CO_2. Total CO_2 and $\delta\ ^{13}CO_2$ was measured on a Picarro G2201 (Picarro Inc). Microbial biomass C was calculated as the difference between chloroform-fumigated and non-fumigated samples scaled by 2.64 (Vance et al., 1987) and litter C-derived microbial biomass was determined using two endmember isotope mixing models.

A sample of roots were separated for root arbuscular mycorrhizal (AM) colonization measurements. To remove pigment, root samples were cleared in 10% potassium hydroxide followed with 85% ethanol to leach excess pigmentation. Roots were acidified in 5% hydrochloric acid and then stained for 5 minutes in 0.05% trypan blue (Comas et al., 2014). AM colonization was determined by suspending root samples in water on a 1x1 cm gridded petri dish and measuring how often arbuscules or hyphae were present at each root-gridline intersect (Giovannetti & Mosse, 1980).

Net mineralization and nitrification

Net N mineralization and net nitrification were measured immediately after ingrowth core harvest. These were expressed as the difference in pools of ammonium (NH^{4+}) and nitrate (NO^{3-}) between an initial sample that was extracted within 24 hours of collection and a sample that was incubated for 2 weeks at room temperature. Inorganic N was extracted from 5 g of soil from each core in 10 mL of 1M KCl solution, and dissolved inorganic N was determined through phenol-hypochlorite and azo-dye colorimetric assays for NH^{4+} and NO^{3-}, respectively (Finzi et al., 1998).

Statistical analysis

To determine the extent to which ingrowth core treatments and fertilization treatments altered the fate of litter C and N amendments, we performed two-way analyses of variance in R version 3.5.1 (R Core Team 2021). Model factors were ingrowth core treatment, fertilization treatment, and their interaction. Post-hoc comparisons between groups were made using the Tukey's HSD test. Differences were considered statistically significant at an alpha level of 0.05 ($p<0.05$) and marginally significant at an alpha level of 0.10 ($p<0.10$). Linear regression was used to investigate the effect of living roots or microbial decomposers on litter C incorporation into MAOM. Data was checked for normality and heteroscedasticity. Outliers, defined as samples where decomposer biomass was greater than 2 standard deviations from the mean, were omitted from linear regression.

Results:

Root impacts on litter C and N transformations did not depend on fertilization

Root ingrowth core treatments and fertilization treatments both altered the fate of litter C and N in SOM, but the root effect did not depend on fertilization. All p-values for ingrowth core treatment x fertilization treatment interactions are above 0.05 (SI Table 2) and root biomass did not vary across fertilization treatment (SI Fig. 4a). As such, subsequent data shown for each factor are aggregated over the other factor.

Root ingrowth reduces litter N remaining in SOM

Root ingrowth did not significantly alter litter C in total SOM (Fig.**2a** , $p>0.10$) but reduced the litter N in total SOM by 20% relative to both root exclusion treatments (Fig. **2b** , $p<0.001$). Within the SOM fractions, root ingrowth reduced both litter C (Fig. **2a** , light green, $p=0.001$) and litter N (Fig.**2b** , light green, $p<0.001$) remaining in the unprotected light POM fraction.

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Root ingrowth alters the balance of C in SOM pools

Of the litter C that remained in SOM, root ingrowth altered the balance of C between SOM pools. Root ingrowth decreased the proportion of litter C remaining in light POM by 32% (Fig. **3a** , $p<0.001$) and increased the proportion of litter C incorporation into protected heavy POM by 30% (Fig. **3b** , $p=0.001$) relative to both root exclusion treatments. Roots did not significantly alter the incorporation of litter C into MAOM (Fig. **3c**). There were no significant differences between fungal only and fungal exclusion treatments.

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Roots mine light and heavy POM for litter N

Root ingrowth selectively mined N from organic matter in both POM pools. Root ingrowth preferentially reduced the litter N remaining in light and heavy POM fractions (Fig. **2b**, green light POM N is 55 % lower with root ingrowth, $p<0.001$; blue heavy POM N is 26% lower with root ingrowth, $p<0.01$). In turn, root ingrowth increased the C:N ratio of litter-derived SOM in light POM (Fig. **4a**, $p<0.001$) and heavy POM (Fig. **4b**, $p<0.001$).

Organic fertilization reduces litter retention in SOM

Organic fertilization reduced litter C and N remaining in the soil relative to control treatments, but there were no significant differences between control and high N fertilization treatments.

Net litter C remaining in SOM was reduced by 14% under the organic fertilization treatment (Fig. **5a** , $p<0.01$) relative to the unfertilized control treatment soils. Within the SOM fractions, the loss of litter C was driven by an 18% reduction in litter C incorporation into MAOM (Fig. **5a** , brown, $p=0.018$). Organic fertilization reduced litter N remaining in total SOM by 12% (Fig.**5b** , $p=0.020$) relative to unfertilized control treatments. Within the SOM fractions, the loss of litter N was primarily driven by a 16% reduction in litter N incorporation into MAOM (Fig. **5b** , brown, $p<0.001$).

Organic fertilization treatments had 25% greater microbial biomass (SI Fig. **4b** , $p=0.09$) relative to unfertilized treatments. Microbial decomposition in organic fertilization treatments was more effective with less litter C remaining in each SOM pool per gram microbial biomass compared to control fertilization (SI Fig. **5, a-c**). However, this decomposition was less effective for litter N than litter C, with no significant

difference in litter N in POM pools per gram of microbial biomass across nutrient treatments (SI Fig. **5, d-e**). Litter C and N incorporation into MAOM was lower per gram of microbial biomass with organic fertilization compared to control fertilization (SI Fig.**5c, 5f**).

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Discussion:

Collectively, this work identifies how roots and soil microbes drive SOM loss and formation in miscanthus systems that can promote soil C sequestration and support plant productivity. Root ingrowth did not promote a net litter C loss from soil (Fig. **2**) despite increased light POM decomposition due to the enhanced transfer of C into heavy POM (Fig. **3**). Notably, we document the potential for roots to mobilize litter-derived N from POM without priming litter C loss (Fig. **2** , Fig. **4**). We also identified that microbial nutrient or carbon limitation may alter how microbes grow and decompose litter-derived SOM, with more litter decomposition and less MAOM formation from litter in organically fertilized soils (Fig.**5**).

It appears that miscanthus roots can mine N from litter without stimulating corresponding litter C losses (Fig. **2**) and can increase the C:N of litter-derived light and heavy POM (Fig.**4**). This raises the question of how miscanthus accesses N from decomposing litter without priming C losses that are commonly observed in other ecosystems (Cheng et al., 2014; Zhu et al., 2014). One plausible mechanism may be that miscanthus roots engineer their rhizosphere microbiome composition or function to preferentially decompose N-rich litter compounds like proteins, potentially by stimulating proteolytic enzyme production (Brzostek & Finzi, 2011). While the specific mechanism remains uncertain, preferential N mining from litter has important implications for miscanthus sustainability (e.g., the propensity of miscanthus to be high yielding and build soil C). The resulting increase in remaining litter C:N may make new litter-derived SOM even more resistant to further decomposition. In addition, there has been a long-standing question of how miscanthus can maintain relatively high yields with limited N inputs (Cadoux et al., 2012). Previous research has posited that high nutrient use efficiency (Beale & Long, 1997) or the promotion of N-fixing symbionts (Davis et al., 2010) sustains N nutrition by miscanthus. Overall, our results suggest that miscanthus may also meet its N nutrition by effectively mining N from litter and SOM.

Our research suggests that roots can actively support the transfer of litter derived C into more protected forms. We observed that the priming of litter decomposition from light POM was balanced by litter C incorporation in heavy POM (Fig. **3**). The composition of heavy POM is not as well-characterized as light POM or MAOM, but this pool is commonly assumed to be composed of stable soil macro- or micro-aggregates (Lavallee et al., 2020). Aggregate occluded SOM is largely formed through root and mycorrhizal symbiont activity (Rillig & Mummey, 2006) and often consists of partially decomposed plant and microbial organic matter fragments. This pool has a higher activation energy for decomposition than low C:N compounds like those in MAOM (Williams et al., 2018) and is more protected from decomposers than free light POM (Keiluweit et al., 2017; Kögel-Knabner et al., 2008). As such, there is an opportunity to build soil carbon in high C:N, heavy POM rather than lower C:N MAOM. The N requirements of low C:N SOM retention have often been cited as a criticism to efforts to use soil C management to mitigate global change (Schlesinger & Amundson, 2019). Future research efforts that investigate how roots can build new, persistent, and high C:N SOM could help realize the potential of soil C sequestration to combat climate change.

We found that the organic fertilizer treatments had the greatest microbial biomass and litter-derived light POM decomposition, in support of our second fertilization hypothesis, but less litter C and N were incorporated into MAOM (Fig. **5** , SI Figs. **4, 5**). On one hand, differences between fertilization treatments could arise from a shift in the microbial community structure or function with organic fertilization (Pan et al., 2014). However, other research at the site has found no significant effects of nutrient treatment on microbial

diversity or mycorrhizal abundance between treatments (Kane et al. 2023,*in review*). On the other hand, C vs. N limitation over microbial decomposition can regulate the rate and efficiency of SOM cycling (Averill & Waring, 2018; Schimel & Weintraub, 2003). As organic fertilization deposits both C and N, our observations could be explained by the alleviation of C limitation and induction of N limitation. In support, we observed a reduction in nitrification rates with organic fertilization relative to unfertilized plots (SI Fig. **6**) and other research found that organic fertilization increases plot-scale microbial respiration (Kane et al., 2023, *in review*). Here, microbial decomposers could increase decomposition and growth while respiring excess C and immobilizing N in living biomass rather than forming more microbially-derived MAOM (Schimel & Weintraub, 2003).

While our experiment identified several important ways living roots and soil microbes control litter decomposition and SOM formation, some mechanisms may not have been fully captured. Our experiment was designed to separate the effects of roots vs. mycorrhizal fungi on litter C and N transformations, but our data only identifies a root effect despite the presence of mycorrhizal fungal symbionts (SI Fig. **7**). The lack of differences between fungal ingrowth and total exclusion cores could be linked to the greater dependence of AM plants on root than hyphal foraging for nutrient uptake (Chen et al., 2016). As such, our experiment may not have isolated fungal effects on litter decomposition and SOM formation. Future efforts should quantify mycorrhizal fungal ingrowth to better investigate the contribution of symbiotic fungi to root-driven SOM transformations. In addition, our observations that fertilization did not impact root biomass (SI Fig **4a**) and that there was no significant interaction between fertilization and ingrowth treatments (SI Table **2**) do not support our first fertilization hypothesis that roots would have the greatest effect in unfertilized soils. While miscanthus root systems do not always respond to fertilization treatments(Amougou et al., 2011), this pattern may have been driven by the stand age of miscanthus in our experiment. These plots were in the third year of growth whereas older, more nutrient limited stands exhibit greater differences in root C allocation and N acquisition (Kantola et al., 2022). As such, future efforts to investigate how nutrient availability alters living root impacts on SOM formation should leverage ecosystems with longer-term fertilization history. Despite these limitations, our data has identified several important mechanisms of SOM formation *in situ* and provides the foundation for future efforts to study how living roots and fungi alter SOM dynamics with more sophisticated measurements, under different environmental conditions, or across different ecosystems and plant-microbe interactions.

This work has expanded our mechanistic understanding of how living roots shape ecosystem processes in agricultural systems. Our finding that miscanthus roots can simultaneously prime N release from litter without an additional C release and transfer C into a more persistent form of SOM has important implications for the sustainability of bioenergy production as well as the viability of restorative agricultural to offset carbon emissions. Overall, our work suggests that living roots can selectively mine N while sequestering soil C. This knowledge can help improve the predictive understanding of SOM cycling that is critical to meeting the goals of restorative agriculture.

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Competing Interests:

The authors declare that they have no competing interests.

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Supplemental Information: