Roots prime microbes to extract nitrogen and stabilize soil organic matter

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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 stable SOM. In part, this conflict is driven by uncertainty in how litter forms new stable SOM with living roots 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 selectively mobilized nitrogen from litter without additional carbon release, and transferred litter carbon into more stable, aggregate-associated SOM. Litter rapidly formed minerally-protected SOM, but native minerally-protected SOM was also rapidly lost. Overall, our fundings suggest that roots stimulate litter decomposition to selectively release nitrogen. We also highlight there may be a rapidly cycling component of minerally-protected SOM that is accessible to roots and microbes.

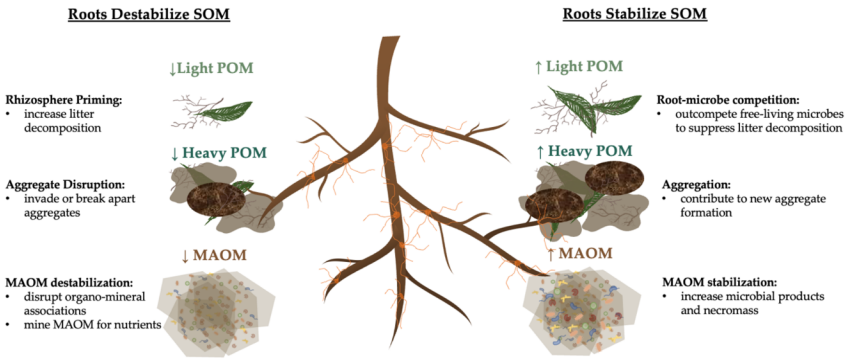
Introduction:

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 destabilize and stabilize 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: 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 stable or protected form of SOM (Cotrufo et al., 2013; Liang et al., 2017). However, MAOM saturates such that accumulation may only be feasible in ecosystems with a current deficit, like degraded agricultural soils (Stockmann et al., 2015). To optimize soil C sequestration in managed ecosystems, understanding what drives the transfer of new litter

inputs between these SOM pools is important for enhancing our predictive understanding of how much soil C can accumulate and how persistent this soil C may be in a changing climate.

Living roots and their associated fungi alter the formation and stabilization of SOM 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 leads to net gains or losses in soil C. In Figure 1, we diagram potential hypotheses for how roots could stabilize or destabilize SOM in the light POM, heavy POM, and MAOM pools through distinct mechanisms. First, root stimulation of microbial decomposition to mineralize soil N can lead to a 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 alter this stabilization by both invading aggregates and increasing the formation rate of new aggregates (Six et al., 2000). Finally, roots can enhance stable MAOM formation by increasing the efficiency of microbial litter decomposition, resulting in greater microbial biomass production and the formation of microbial necromass. This necromass can associate with mineral surfaces and is the main precursor to MAOM in grassland ecosystems (Angst et al., 2021; Liang et al., 2017). However, roots may also destabilize 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 stabilization in these different pools.



The extent to which roots and mycorrhizal fungi facilitate SOM stabilization or destabilization 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). In the absence of roots and tightly coupled root-microbe interactions, the degree to which fertilization alters SOM cycling depends upon the activity of 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 more stable SOM. Collectively, the effect of fertilization on SOM stabilization 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 stabilization in distinct SOM pools and 2) assess**

how SOM stabilization processes are 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 stabilization would be strongest in unfertilized soil and (2) organic fertilizer would accelerate microbial decomposition and SOM cycling to a greater extent than inorganic fertilizer. 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, that excluded roots but were open to fungal ingrowth, or that excluded both roots and fungi to quantify the effect of living roots and fungi on new SOM formation (SI Fig. **1**). 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 more stable, heavy POM. Roots also selectively mobilized litter N from both POM pools without additional C release. As such, roots can promote the net retention of more stable C while still enhancing N mining. These root effects did not depend on fertilization. However, organic fertilization enhanced microbial decomposition of litter without increasing litter stabilization in MAOM. Regardless of treatment, the rapid stabilization and destabilization of MAOM in our ingrowth cores supports recent theories that MAOM may cycle dynamically.

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 $5m^2$ and was established by planting 25 miscanthus rhizomes using $1m^2$ grid spacing (site map, SI Fig. **2a**). 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). For this experiment, we utilized the control, high-level inorganic, and organic fertilization treatments.

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 exclusion and fungal ingrowth, and root and fungal exclusion) and 3 fertilization treatments (no fertilization, high-level inorganic fertilization, or organic fertilization). 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, resulting 6 cores per plot (SI Fig. **2b**). This resulted in a total of 90 experimental ingrowth cores (3 cores x 3 fertilization treatments x 5 plots x 2 plants/plot).

Ingrowth core construction and installation

Ingrowth core treatments included root and fungal ingrowth, root exclusion and fungal ingrowth, and root and fungal exclusion (SI Fig.**1**). Each ingrowth core was constructed with 10cm long, 4.5cm diameter

rigid plastic 5mm mesh tubing. The top 2.5cm of each core was inserted into 5cm long PVC collars and attached with elastic sealant. Mesh bases were sewn onto each core with 12lb nylon fishing line and each core was wrapped with mesh that was glued on with 100% silicon adhesive. Root and fungal ingrowth cores were constructed with 1.5mm polyacrylic mesh that allowed fine root ingrowth. Root exclusion cores were constructed with 50um nylon mesh that was too fine for root ingrowth but allowed hyphal ingrowth (Phillips et al., 2012). Root and fungal exclusion 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.**1**).

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 10cm were collected from each future ingrowth core location and were brought back to the lab where they were sieved to 2mm 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 53um diameter in a 9:1 soil:sand ratio. Isotopically enriched corn leaf litter, generated as described in Ridgeway et al., 2022, was used as the substrate in each ingrowth core. This litter had a %C of 41.7% (±0.17%), C:N of 18.8 (±0.64), δ ^{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 250 mg labeled litter was gently mixed in to the top 2cm.

Within 5 days of initial soil collection, the assembled cores were transported to the field location where they were installed into the top 10cm of soil in each corresponding treatment plot (SI Fig. **2a**). 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. **2b**). 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, isotope analysis, and litter C and N fate

Ingrowth cores were destructively harvested in September and litter C and N inputs were traced into SOM pools (Ridgeway et al., 2022). A 5g 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 1.85g/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 53um sieve.

To trace the fate of ^{13}C and ^{15}N litter amendments, the soil fractions were analyzed for %C, %N, δ^{13}C, and δ ^{15}N using a Thermo Fisher Delta V+ isotope ratio mass spectrometer interfaced with a Carlo Erba NC2500 Elemental Analyzer. Two endmember mixing models were used to determine the proportion of C and N in each soil fraction that came from native SOM vs. the enriched litter substrate (Derrien & Amelung, 2011).

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 δ $^{13}CO_2$ 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-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 1x1cm 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 5g of soil from each core in 10mL 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. Outliers, defined as samples where decomposer biomass was greater than 2 standard deviations from the mean, were omitted from linear regression.

Results:

Both ingrowth treatments and fertilization affect litter C and N transformations, but there was no interaction between these factors.

Root ingrowth core treatments and fertilization treatments both altered litter C and N transfers between SOM pools, but the root effect did not depend on fertilization (SI Table 2, all p-values for ingrowth core treatment x fertilization treatment interactions are above 0.05). 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 remaining in total SOM (Fig. **2a** , $p>0.10$) but reduced the litter N remaining 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 pols. Root ingrowth decreased the proportion of litter C remaining in light POM (Fig. **3a** , $p<0.001$) and increased the proportion of litter C incorporation into protected heavy POM (Fig. **3b** , $p=0.001$). Roots did not significantly alter the incorporation of litter C into MAOM (Fig. **3c**).

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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.

Organic fertilization reduced the litter C remaining in total SOM by 14% (Fig. **5a** , $p<0.01$) relative to the unfertilized control treatment. 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. **3** , $p=0.09$) 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. **4 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. **4, 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.**4c, 4f**).

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MAOM formation does not increase with more decomposers

We observed weak but significant negative correlations between the proportion of litter C in MAOM and microbial biomass (Fig. **6a** , $p<0.001$). Root biomass was also negatively correlated with the proportion of litter C in MAOM (SI Fig. **5**), but did not significantly alter native MAOM and did not covary with microbial biomass (SI Fig. **6**). We also observed the destabilization of native MAOM after field incubation compared to the initial soil mixtures. Over the course of the growing season, we observed the loss of approximately 15% of native MAOM (Fig. **6b** , $p<0.10$ for all soil types).

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Discussion:

Collectively, this work identifies critical mechanisms of root and microbial control over SOM stabilization and destabilization in miscanthus systems that can promote soil C sequestration and support plant productivity. Root ingrowth did not lead to a net litter C loss (Fig. **2**) despite increased litter decomposition due to

enhanced transfer of C into more stable SOM (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 stabilization in organically fertilized soils (Fig. **5**). Across all treatments, litter-derived OM was rapidly incorporated into MAOM and appeared to reflect greater microbial processing despite declining with increasing microbial biomass (Fig.**6**), supporting recent evidence that MAOM may be more dynamic than previously thought.

It appears that miscanthus roots can mine N without priming soil C losses (Fig. **2**) and 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. Here, we show that highly effective N mining may drive the high productivity, low fertilization demand, and soil C sequestration of miscanthus cultivation (Dohleman & Long, 2009; Heaton et al., 2008; Smith et al., 2013).

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 stabilization 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 for stabilizing 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, stable, and high C:N SOM could help realize the potential of soil C sequestration to combat climate change.

Organic fertilization may promote saprotrophic soil microbes that more effectively decompose litter substrates and less efficiently form more stable heavy POM and MAOM. We found that the organic fertilizer treatments had the greatest microbial biomass and light POM decomposition, in support of our second fertilization hypothesis, but less litter C and N were incorporated into MAOM (Fig. **5** , SI Figs. **3, 4**). 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. **7**) 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).

Our research supports recent theory that some component of MAOM may dynamically cycle (Kleber et al., 2021; Sokol et al., 2022). Soil C persistence theory has evolved to rely on two fundamental assumptions, that (1) microbial growth and necromass production are tightly coupled to the stabilization of microbially-derived MAOM and (2) that MAOM is physically inaccessible to decomposers, leading to slow turnover rates and long-term persistence (Cotrufo et al., 2013; Lehmann & Kleber, 2015). While our MAOM appeared to reflect greater microbial processing due to a lower, constrained C:N (Fig. **4c**), microbial biomass was weakly negatively, rather than positively, correlated with MAOM formation (Fig. **6a**). In contrast to the first assumption, this pattern supports recent findings that microbial growth and efficiency may not drive MAOM stabilization (Craig et al., 2022; Sokol et al., 2022). In contrast to the second assumption, the rapid transfer of litter carbon into MAOM (Fig. **2**) and the loss of native MAOM over a single growing season (Fig. **6b**) suggest that some part of MAOM may turnover rapidly. Further, declining MAOM formation with microbial and root biomass (Fig. **6a** , SI Fig. **5**) support recent findings that MAOM can be continually processed by microbes (Fossum et al., 2022) and that roots can destabilize MAOM (Jilling et al., 2021; Li et al., 2021). However, other studies observe that microbial growth efficiency promotes MAOM formation (Kallenbach et al., 2016; Ridgeway et al., 2022) and that MAOM generally persists longer than POM pools (Heckman et al., 2022). As such, continuing research should be directed at determining where and under what conditions MAOM cycles dynamically.

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. **8**). However, 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). Future efforts should quantify mycorrhizal fungal ingrowth to better investigate the contribution of symbiotic fungi to root-driven SOM transformations. In addition, our observations of no significant interaction between fertilization and ingrowth treatments (SI Table 1) do not support our first fertilization hypothesis that roots would have the greatest effect in unfertilized soils. However, this pattern may have been driven by the stand age of miscanthus. 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 stabilization *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 build stable soil C has important implications for the sustainability of bioenergy production as well as the viability of restorative agricultural to offset carbon emissions. Our findings also have important implications for our understanding of the formation and stability of mineral bound carbon and nitrogen. The lack of a positive correlation between microbial and root biomass with MAOM formation, the rapid transfer of litter into MAOM, and the loss of native MAOM suggest that the mechanisms that control MAOM formation and loss are more dynamic than current theories assert (Heckman et al., 2022; Kleber et al., 2021; Sokol et al., 2022). Overall, our work suggests that living roots can mine N while stabilizing 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:

SI Figure 1: Ingrowth core experimental design

SI Figure 2: Site map, fertilization treatments, ingrowth core placement example

SI Table 1: replication for each treatment

SI Figure 3: Microbial Biomass by nutrient treatment

SI Figure 4: litter C and N in light POM, Heavy POM, and MAOM per microbial biomass

SI Figure 5: Root ingrowth vs. litter C in MAOM

SI Figure 6: Root vs. microbial biomass

SI Figure 7: Net nitrification between fertilization treatments

SI Figure 8: Root mycorrhizal colonization example

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