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Mechanisms of soil organic matter persistence vary across time and soil depth in long-term cropping systems of the North Central US

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ABSTRACT

Soil organic matter (SOM) declines under agricultural production have been well documented, despite efforts to maintain or enhance SOM through practices like rotational diversity and increased carbon (C) input quantity and quality. However, a critical knowledge gap remains in understanding how system management alters the microbial processes that drive C input turnover and stabilization across time and soil depths. This study addresses this gap by leveraging a long-term cropping systems trial to investigate microbial mechanisms of SOM turnover and stabilization across a representative range of cropping systems in the North Central US. We assessed microbial and chemical soil characteristics at two key sampling times throughout the growing season and linked these measurements to indicators of SOM persistence. Particulate organic matter (POM) C:N exhibited significant ($P < 0.001$) variation across cropping systems, depth, and time reflecting a gradient of system C input quality. POM-C was greatest in pasture systems ($P < 0.001$) at both time points, suggesting stability of the relationship between POM inputs and decomposition across the growing season. Additionally, microbial growth and respiration were highest in pasture ($P > 0.001$), which was consistent across time, indicating an active microbial community that facilitates SOM turnover and stabilization. Our findings provide novel insights on the role of rotational and plant input diversity for enhancing microbial turnover and slowing SOM decline through POM substrate quality, particularly in pasture systems, across time and soil depths. This research will serve to inform future cropping system-level soil management strategies aimed at improving SOM persistence.

1. Introduction

The loss of soil organic matter (SOM) due to agricultural production remains a systemic problem in much of the Northcentral United States (Chaplot and Smith, 2023; Grandy and Robertson, 2007). Stabilizing or even reversing these losses will require significant efforts to slow land degradation and reduce the extractive nature and intensity of modern commodity crop farming (Janzen, 2006; Wiesmeier et al., 2016). A significant portion of agriculture in the Northcentral region occurs on Mollisols, soils rich in SOM and highly valued for their productivity. They account for > 25 % of global farmland with significant cultivated acres in the U.S., Eastern Europe, China, and Argentina (Liu et al., 2012; Xu et al., 2020). Since the conversion of native prairies to cropping systems, in the mid-nineteenth century, it's estimated that over 50 percent of the SOM in Northcentral Mollisols has been lost (Posner et al., 2008; Xu et al., 2020). This equates to C loss rates ranging from 1.6 to 3.7 Mg C ha^{-1} yr^{-1} (Huggins et al., 2007), or annual SOM losses between 3.2 and 7.4 Mg SOM ha^{-1} yr^{-1}, with soil organic carbon (SOC) making up roughly 50 % of SOM (Pribyl, 2010). Best management practices touted to support SOM persistence including increasing plant and animal C inputs, cropping system diversification, and perennial and/or legume crop inclusion, have yielded inconsistent results (Diederich et al., 2019; Dietz et al., 2024; McDaniel et al., 2014; Sanford et al., 2012). However, SOM persistence is not just a function of C inputs. It is also governed by microbial communities, which regulate C input flow into stable soil SOC fractions as necromass or as organo-mineral associations (Buckeridge et al., 2020; Cotrufo et al., 2013; Tao et al., 2023). A knowledge gap exists in our understanding of how the microbial processes responsible for SOM persistence and turnover—such as microbial decomposition, C assimilation, and necromass contributions—

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are influenced by agricultural management practices and fluctuate across growing seasons. Assessing the temporal variation of microbial communities and their functions related to SOM formation will provide a nuanced understanding of how cropping systems can build or maintain SOM, ultimately promoting a more sustainable future for agricultural management.

Both the quantity and quality (C:N) of C inputs contribute to stable SOM formation via their control on microbial decomposition rates and efficiency of C assimilation into microbial biomass (Cotrufo et al., 2013; Xie et al., 2022). One prominent current conceptual framework considers SOM as two functionally distinct fractions: particulate organic matter (POM), considered primarily plant-derived and readily subject to microbial decomposition; and mineral-associated organic matter (MAOM), considered to be derived from microbial products and labile root C exudates, and a stable fraction of SOM (Lavallee et al., 2020; Moni et al., 2012). The quality and quantity of plant inputs to soil should be directly reflected in POM-C and POM C:N. Given POM serves as microbial substrate, it may regulate the physiological diversity and growth of microbial communities, promoting more efficient stabilization of labile C inputs via microbial turnover and necromass contributions (Buckeridge et al., 2020; Haddix et al., 2016; Lehmann and Kleber, 2015).

In theory, agricultural systems that include a larger quantity of high-quality, low C:N ratio C inputs like legume perennials and/or animal manures would promote greater microbial biomass and growth, thereby increasing microbial necromass-derived MAOM (Kallenbach et al., 2016, 2015; Potter et al., 2022; Six et al., 2006). Total microbial necromass contributions may contribute $>50\,\%$ of persistent SOC in agricultural soils and tend to reflect the relative abundance of fungal: bacterial biomass (Kallenbach et al., 2016; Liang et al., 2019; Zhu et al., 2024). Amino sugars, which serve as necromass biomarkers, provide insight towards the relative contributions of fungal (glucosamine (GluN)) and bacterial (muramic acid (MurA)) necromass to stable SOC (Guggenberger et al., 1999; Liang et al., 2019). Assessment of microbial necromass biomarkers may help describe how cropping system management alters microbial contributions to SOM. However, further research across time, cropping system, and soil depth is needed to refine our understanding of how these interactions operate in agricultural contexts.

Previous research has provided valuable insight into how management strategies affect relationships between plant C inputs and microbial function/contributions to persistent SOM in agricultural systems (Bardgett and van der Putten, 2014; Kallenbach et al., 2019; Six et al., 2006). Practices which increase rotational diversity have been shown to support greater microbial carbon use efficiency (CUE), degradative enzyme activities, microbial abundance, and necromass contributions to SOC, even under scenarios of reduced C inputs (Kallenbach et al., 2015; King and Hofmockel, 2017; McDaniel et al., 2014). Specifically, systems with higher quality (lower C:N) inputs contributed to significant increases in POM-C (Cates et al., 2016; Jokela et al., 2011; Liptzin et al., 2022) and other biological indicators of labile C like potentially mineralizable carbon (PMC) (Diederich et al., 2019; Liptzin et al., 2022). These system-level changes have also been reported to increase microbial biomass abundance (King and Hofmockel, 2017), fungal richness, and overall microbial diversity (Potter et al., 2022). Another recent system-level study showed that microbial CUE was higher in diversified systems, but gains in SOM were ultimately offset by an increase mineralization of MAOM (Rui et al., 2022). While the outcomes of these studies have benefited our understanding of microbially mediated SOM dynamics, the seasonal variation of biological measurements including CUE, microbial growth, PMC, and phospholipid fatty acid analysis (PLFA) have recently been highlighted (Adingo et al., 2021; Guo et al., 2022; Schnecker et al., 2023), suggesting that an understanding of plant-soil-microbe dynamics across seasons is needed to advance understanding on cropping system impacts on SOM sequestration and persistence (Leitner et al., 2021).

The overarching goal of this work is to determine the consistency of microbial mechanisms of SOC turnover and stabilization across a range of agricultural management practices with altered C input quantity and quality at two time points and two soil depths across a growing season. This work was conducted at the Wisconsin Integrated Cropping Systems Trial (WICST), established in 1990, which includes six cropping systems along a gradient of cash-grain, dairy-forage, and grazed pastures that are broadly representative of agricultural practices found throughout the Northcentral U.S. Previous work at this site showed that these distinct management of these systems may have impacted the quantity and quality of C inputs, as well as microbial physiology and overall microbial diversity (Cates et al., 2016; Diederich et al., 2019; Rui et al., 2022; Potter et al., 2022).

In this study, we aim to advance the work of previous findings by investigating the temporal and depth-related changes in microbial structure and function, as well as their connections to management practices and C inputs. Our two objectives are to (i) determine the effects of cropping system, sampling time, and depth on microbial growth, CUE, microbial community composition, C storage within soil fractions, and microbial necromass contributions and (ii) Identify significant correlations between response variables using Pearson's correlations. We hypothesized that: (i) cropping systems with lower C:N plant and animal inputs will accumulate greater POM-C, and the quality of the inputs will be reflected in the POM C:N; (ii) Microbial CUE, absolute abundance of fungi, and F:B biomass will be higher in more perennialized systems with energetically favorable low C:N inputs; (iii) differences in soil biological and chemical characteristics across cropping systems will also be transient across the growing season and soil depths; and (iv) necromass contributions to soil organic matter will reflect the active microbial community, with increased fungal necromass contributions in systems with the highest absolute abundance of fungi and F:B biomass.

2. Materials and methods

2.1. Site description and sampling

The Wisconsin Integrated Cropping Systems Trial (WICST) is a long-term cropping systems experiment, established in 1990 and located at the University of Wisconsin- Madison Arlington agricultural research station (Arlington, WI) (43°170 4500 N, 89°220 4800 W). Soils at the site are classified as Plano silt loam (fine-silty, mixed, superactive, mesic Typic Argiudoll, US Department of Agriculture [USDA] Soil Taxonomy) with 6 % sand, 72 % silt, and 22 % clay.

WICST is arrayed in a randomized complete block design with six cropping systems ("treatments") and four blocks (replicates). Every phase of each cropping system is present in each block every year (Fig. 1). The six systems are composed of three grain-based (Maize, MS, organic MSW) and three forage based (MAAA, organic Mo/AA Pasture) systems (see Sanford et al., 2012 for complete description). Plots are 0.3 ha, with commercial farm-scale equipment used for all fieldwork.

The two sampling times (mid and late-season) were selected to capture temporal variations in microbial processes related to SOC turnover. Mid-season (July) was selected, as it typically coincides with peak plant growth and rhizodeposition, which can heavily influence microbial. In contrast, late-season (September) was selected as it reflects a decline in plant growth and C inputs, which, in turn, may lead to a decrease in cropping system-associated differences in microbial SOC turnover and stabilization. For July of 2022, average daily highs and lows were 28 and 16°C, respectively, with approximately 105 mm of rainfall recorded. Maize was in the V12 developmental stage during July sampling. For September of 2022, average daily highs and lows were 23 and 10°C, respectively, with approximately 90 mm of rainfall recorded. Maize was in the R3 milk stage during September sampling. All samples were taken in the maize phase of all systems except the pasture. Sampling was performed by removing 10 soil cores (30 cm) in a zig-zag pattern to equally sample across maize rows. Each core was divided

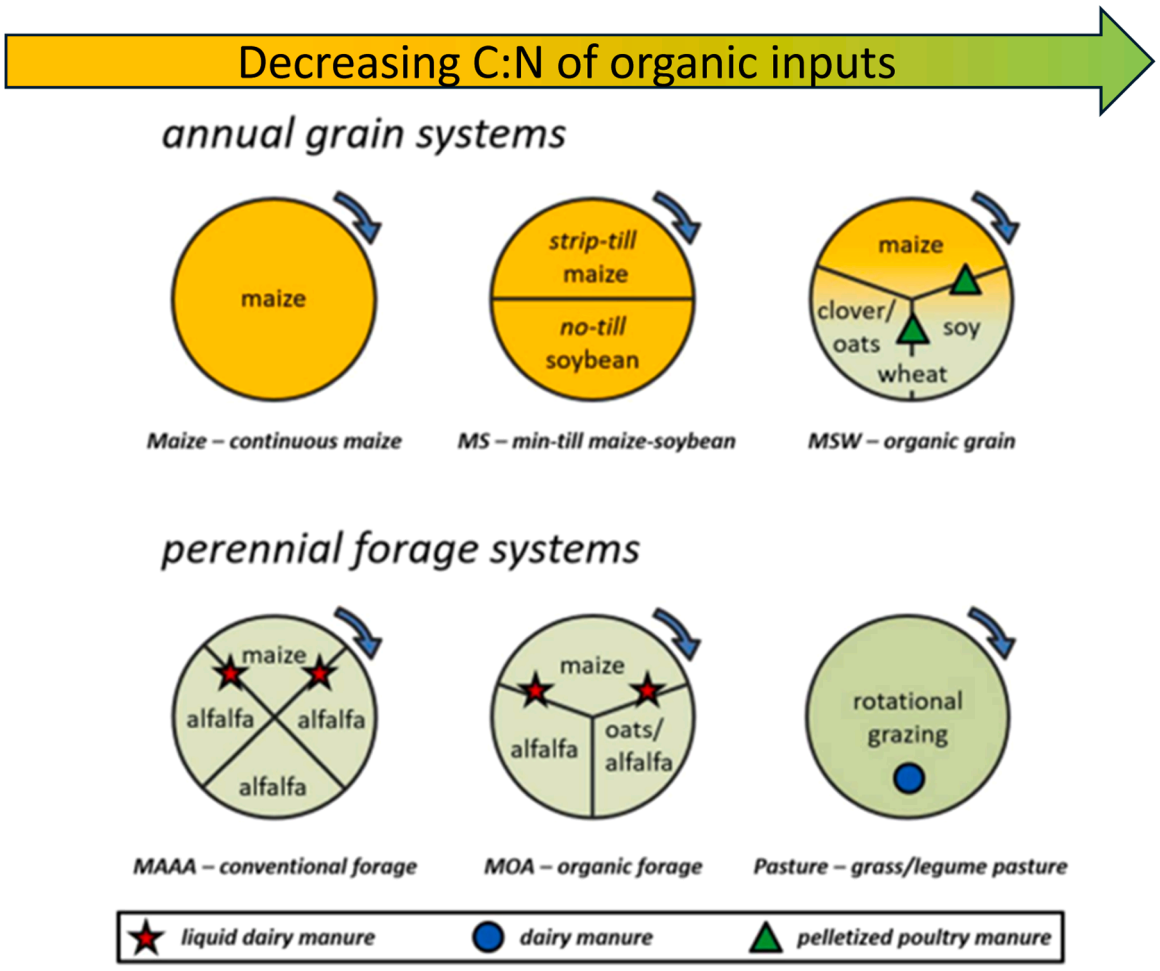

Fig. 1. The six cropping systems at the Wisconsin Integrated Cropping Systems Trial (WICST).

between 2 soil depth intervals (0–15 and 15–30 cm). Two soil depth layers (0–15 and 15–30 cm) were selected for analysis to align with previous site research which has shown soil characteristics dramatically differ between these depths (Diederich et al., 2019). The 0–15 cm depth, which typically supports the highest levels of microbial activity and turnover, may be most sensitive to system management and seasonal changes, while the 15–30 cm depth provides insight into how microbial communities are linked to legacy SOC persistence and storage.

The divided soil cores were then homogenized by depth and plot and kept on ice for storage and transport to the lab. Upon return to the lab, soils were sieved (2 mm) and a portion was kept at 4°C up to one week for CUE analyses, another was freeze-dried and maintained at −20°C for PLFA analysis, and the remainder was air-dried for amino sugar analysis, SOM fractionation, and CN analysis. Amino sugars and SOM fraction data was collected at both soil depths and sampling times. CUE, MGR, and respiration was estimated for the 0–15 cm depth only at both sampling times, and PLFA was estimated at the mid-season sampling time only at both soil depths.

2.2. ^{18}O carbon use efficiency (CUE)

2.2.1. ^{18}O-DNA and respiration measurements

To directly compare community level CUE across cropping systems during mid and late-season, microbial CUE was determined by the substrate independent ^{18}O method as described by Spohn et al. (2016). 22 g of fresh soil was weighed into a beaker and adjusted 45 % of its WHC with DI water. After allowing the soil and water to equilibrate for 1 hour, 15 g of this soil was added to a 473 mL mason jar to measure respiration. 300 mg of the initial soil pool was also added to 2 mL microcentrifuge tubes. ^{18}O water was added to one set and DI water was added to another set as an unlabeled control, analyses were performed in triplicate. After adding soil, microcentrifuge tubes with open lids were placed inside 15 mL falcon tubes with closed lids. The remainder of the initial 22 g of soil was oven-dried to confirm the GWC. Both the mason jars and microcentrifuge tubes were placed in the dark at room temperature for a 72-hour pre-incubation period.

After pre-incubation, soils were adjusted to 65 % WHC based on their current GWC with DI water. To estimate respiration, gas samples were taken using a 10 mL syringe and injected into an infrared gas analyzer (IRGA) for CO_2 analysis (T0) (EGM-5, PP Systems, USA). Gas samples were also taken at 24 and 48 hours (T1 and T2). Jars were left open to equilibrate with room atmosphere for 30 minutes prior to the T0 sample and after the T1 sample.

Microcentrifuge tubes were simultaneously adjusted to 65 % WHC using 20 at% ^{18}O-H_2O or DI water. All tubes were incubated inside the 15 mL falcon tubes for 48 hours. Incubations were terminated by closing microcentrifuge lids and placing in a −80°C freezer until DNA extraction.

DNA extractions were performed using a DNeasy PowerSoil Pro Kit (Qiagen, USA) following manufactures protocol with a 15-minute initial vortex time. Molecular grade water was substituted for the elution buffer in the final step, as the elution buffer contains oxygen compounds that interfere with ^{18}O analysis. DNA was then quantified on an Invitrogen Qubit Flex fluorometer (ThermoFisher Scientific, USA). DNA quantified was assumed to contain 31.21 % O for sample preparation (Qu et al., 2020).

DNA extract was added and dried in 5 × 3.5 mm silver capsules, spiked with sucrose to meet minimum level of detection for total O analysis and sent to the UC Davis Stable Isotope Facility for total O and ^{18}O analysis. An additional set of sucrose-only samples were prepared for analysis as controls.

2.2.2. ^{18}O CUE calculations

Following $\delta^{18}O$ quantification of DNA and sucrose-only controls, the **^{18}O of DNA** was calculated for both the control and enriched samples using the following equation derived from Kallenbach et al. (2015):

$$\frac{(\delta^{18}O_{sample} - F_s \times \delta^{18}O_{sucrose})}{F_{DNA}} = 18O \text{ of DNA}$$

F_s and F_{DNA} are the mass fractions of sucrose and DNA, respectively, and $\delta^{18}O_{sucrose}$ is the $\delta^{18}O$ of sucrose relative to an internal ^{18}O standard.

The **total new DNA produced (μg g^{-1} dry soil)** during incubation was calculated as follows:

$$\frac{CDNA * (\delta 18O\text{enriched} - \delta 18O\text{control})}{(\delta 18OH2O - \delta 18O\text{control})} = DNA\text{produced } (\mu g\ g - 1 \text{ dry soil})$$

C_{DNA} is the average concentration of DNA measured between control and enriched samples (ug DNA g^{-1} dry soil). $\delta^{18}O_{enriched,\ control,\ \&\ H2O}$ is the $\delta^{18}O$ of the enriched and control samples, respectively, and of the final soil water (20 at%).

$DNA_{produced}$ was transformed into **microbial biomass C (MBC) produced (ug g^{-1} dry soil)** using the following linear regression equation between the concentration of DNA and MBC (Chen et al., 2020):

$$MBC\text{produced} = 16.33 \times DNA\text{produced} + 4.85$$

$MBC_{produced}$ is the total amount of MBC produced over the incubation period. **Microbial growth rate (MGR; ng C g^{-1} dry soil hr^{-1})** was then calculated as follows:

$$MGR = \frac{(MBC\text{produced} * 1000)}{T}$$

T is the incubation time in hours (48). 1000 is used as a multiplier to move from μg to ng.

Microbial Respiration ($C_{respiration}$; ng C g^{-1} dry soil hr^{-1}) was calculated from an average of the two 24-hr periods (T1 &T2) in which CO_2 was measured via IRGA. The T0 time point (ambient CO_2; ppm) was subtracted from the averaged value).

$$C\text{respiration} = \frac{((C - C0) \times M \times V \times 298)}{(Vm \times DW \times 273 \times T)}$$

C and C_0 represent the average 24 hr CO_2 production values and the initial ambient CO_2 concentration, respectively. M is the molecular mass of C; V is the volume of the headspace in the mason jars; 298 is the incubation temperature (K); V_m is the standard molar volume of ideal gas (L/mol); DW is the dry mass of soil (g); 273 is the absolute temperature (K) at the standard atmospheric pressure; T is the incubation time in hours.

Microbial CUE was calculated using the following equation (Spohn et al., 2016):

$$CUE = \frac{MGR}{(C_{respiration} + MGR)}$$

2.3. PLFA

The soil microbial biomass and community structure was assessed using phospholipid fatty acids (PLFA) analysis. 2.5 g of freeze-dried finely ground soil was prepared, then membrane lipids of soil microbes were extracted and purified prior to separation by silicic acid chromatography. A Hewlett-Packard 6890 gas chromatograph (Agilent Technologies, USA) was used for identification and quantification of extracted PLFAs. Total nmol lipid g soil^{-1} was used as an index of microbial biomass and the relative abundance of specific lipids was determined by calculating the moles of a given lipid/total moles lipid per sample (mol%). The mol% of chemically similar fatty acids were then combined into groups representing specific groups within the microbial community or "guilds".

2.4. Microbial necromass characterization

To determine the effects of cropping systems on contributions of microbial necromass to SOC, we assayed amino sugars specific to bacterial and fungal necromass following the method described by Rui et al. (2022). 1 g of air-dried soil was finely ground and hydrolyzed with 6 M HCl at 105 °C for 8 h to release the amino sugar monomers. Samples were then filtered, dried with a rotary evaporator, and redissolved in DI water. After purification, derivatization agents were added to residues, which transformed amino sugars into aldononitrile derivatives that were extracted with 1.5 mL dichloromethane from the aqueous solution. Finally, amino sugar derivatives were analyzed with an Agilent 6890 GC (Agilent Technologies, USA) equipped with a J&W Scientific Ultra-2 column (25 m × 0.2 mm × 0.33 μm) and flame ionization detector. The individual amino sugar derivatives were identified by comparing them to authentic standards.

2.5. SOM physical fractionation

To determine the effects of cropping system on functionally distinct SOM fractions, soil was fractionated to POM and MAOM. Initially, aggregates were dispersed from 10 g of air-dried soil with 30 mL of 5 % sodium hexametaphosphate solution. Samples were shaken for 18 hours at 130 RPM. After shaking, fractionation was performed using a Fritsch automatic wet sieve shaker (Fritsch, Idar-Oberstein, Germany) at an amplitude of 1.5 Hz for 1 minute. Soil that passed through the 53 μm sieve was operationally defined as MAOM. Soil > 53 μm was operationally defined as POM. The dry mass of each fraction was recorded, and fractions were subject to a ball mill grinder prior to combustion CN analysis.

2.6. Statistical analysis

A linear mixed-effects ANOVA was used to determine the effects of sampling time, depth, and cropping system on SOM fractions and necromass biomarkers; the effects of sampling time and cropping system on microbial CUE; and the effects of depth and cropping system on PLFA absolute abundance and microbial group ratios (Objective 1). Blocks were set as a random effect. Significant effects between system treatment means were assessed with a Tukey-HSD post hoc test ($p < 0.05$). In general, individual treatment effects are presented in tables, and interaction effects are presented in figures. All response variables were included in Pearson's correlation analysis to identify significant correlations (Objective 2). By assessing relationships between those inherent difference and response variables, inferences can be made on how cropping systems management can improve SOC stabilization to build or at least maintain SOC. All statistical analyses were performed in R studio (v.2023.03.0) using packages nlme and corrplot (R Core Team, 2022).

3. Results

3.1. Contributions of plant C inputs to SOM quantity and quality

The mass of POM-C significantly varied across systems, however this effect differed across depths (Fig. 2; Table 1; System×Depth $P < 0.05$). For example, at 0–15 cm, pasture supported significantly higher POM-C by 67–128 % across both sampling times compared to all other cropping systems; this was not observed at 15–30 cm. At 15–30 cm, POM-C was

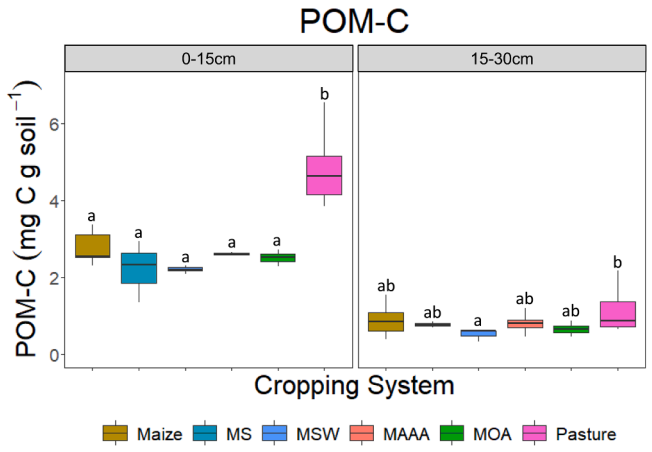

Fig. 2. POM-C content at 0–15 cm and 15–30 cm in WICST. Vertical lines indicate the standard errors. The different letters indicate significant differences between the six cropping systems within their respective depth interval ($p < 0.05$). Maize; MS, maize and soybean; MSW, maize, soybean, and wheat; MAAA, maize, alfalfa, alfalfa, alfalfa; MOA, maize, oats/alfalfa, alfalfa; Pasture.

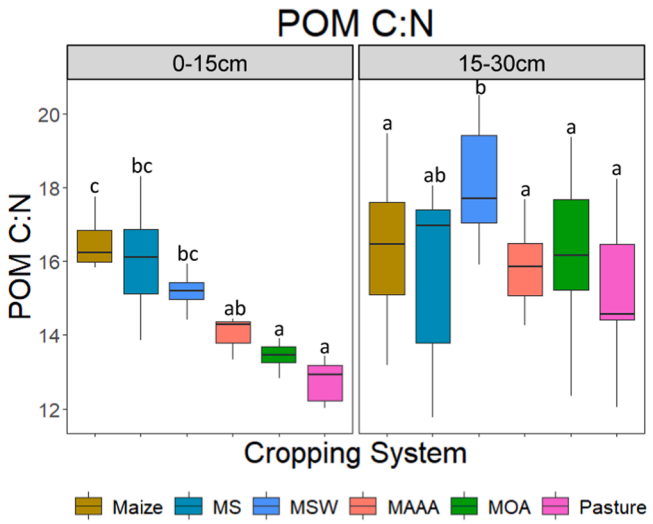

Fig. 3. POM C:N at 0–15 cm and 15–30 cm in WICST. Vertical lines indicate the standard errors. The different letters indicate significant differences between the six cropping systems within their respective depth interval ($p < 0.05$). Maize; MS, maize and soybean; MSW, maize, soybean, and wheat; MAAA, maize, alfalfa, alfalfa, alfalfa; MOA, maize, oats/alfalfa, alfalfa; Pasture.

greater in pasture compared to MSW, and no other differences were observed between cropping systems. POM-C was significantly lower at 15–30 cm than at 0–15 cm across both sampling times by > 200 %. There was no effect of sampling time on POM-C.

POM C:N also significantly varied across systems, however, similar to POM-C, this effect differed across depths (Fig. 3; Table 1; System×Depth $P < 0.05$). For example, at 0–15 cm, POM C:N of MAAA, MOA, and Pasture were significantly lower than Maize and MS across both sampling times, but this was not observed at 15–30 cm. At 15–30 cm, POM C:N was greater in MSW compared to all other systems except MS. POM C:N significantly varied by depth. However, this effect differed by time (Time×Depth $P < 0.005$). At 15–30 cm, POM C:N was 16 % higher during late-season compared to mid-season, but this was not observed at 0–15 cm.

MAOM-C was significantly higher in Pasture compared to MSW, but on average was 4–30 % higher than all other systems (Table 1; System $P < 0.05$). No other significant differences in MAOM-C were observed between cropping systems. MAOM-C also significantly varied across depth (Depth $P < 0.05$), being higher at 0–15 cm than 15–30 cm by 31 %.

MAOM C:N significantly varied across depth (Table 1; Depth $P < 0.05$), being higher at 15–30 cm than 0–15 cm by 3 %. MAOM C:N was similar across all systems at both depths. MAOM C:N was marginally significant across cropping systems (Table 1; System $P = 0.054$). There was no time effect for MAOM C:N.

3.2. Cropping system effects on microbial composition and function

Total PLFAs, as well as the specific PLFA abundance of microbial groups including bacteria, fungi, AM fungi and actinomycetes significantly varied across cropping systems (Fig. 4; Table 2; System $P < 0.05$). For example, Pasture supported significantly higher total PLFA across both depths compared to MS, MSW, and MOA. Pasture also supported significantly higher PLFA abundance of actinomycetes, AM fungi, and total fungi than all other systems. F:B of Pasture was significantly higher than Maize, MS, MSW, and MOA. Total PLFA, bacteria, Gram-negative, and Gram-positive bacteria were similar between Maize and Pasture. F:B

Table 1
SOC and C:N of POM and MAOM by cropping system, time, and depth at WICST. Different letters indicate significant differences across cropping system or depth interval ($p < 0.05$). Maize; MS, maize and soybean; MSW, maize, soybean, and wheat; MAAA, maize, alfalfa, alfalfa, alfalfa; MOA, maize, oats/alfalfa, alfalfa; Pasture.

System	POM-C (mg g^{-1})	POM C:N	MAOM-C (mg g^{-1})	MAOM C:N
Maize	1.80 ± 0.14a	16.4 ± 0.48c	19.5 ± 1.57ab	12.1 ± 0.19
MS	1.48 ± 0.17ab	16.2 ± 0.53bc	19.7 ± 1.59ab	12.0 ± 0.19
MSW	1.32 ± 0.15b	16.8 ± 0.47c	15.8 ± 1.57a	11.6 ± 0.19
MAAA	1.72 ± 0.16ab	15.0 ± 0.47ab	17.6 ± 1.57ab	11.6 ± 0.19
MOA	1.61 ± 0.14ab	14.9 ± 0.47ab	18.9 ± 1.59ab	11.7 ± 0.19
Pasture	3.01 ± 0.14c	14.0 ± 0.48a	20.6 ± 1.62b	11.6 ± 0.20
Time				
Mid-Season	1.92 ± 0.12	14.8 ± 0.40a	19.0 ± 1.37	11.8 ± 0.16
Late-Season	1.72 ± 0.12	16.3 ± 0.41b	18.4 ± 1.37	11.7 ± 0.16
Depth				
0–15 cm	2.86 ± 0.12a	14.7 ± 0.40a	21.2 ± 1.36a	11.5 ± 0.16a
15–30 cm	0.78 ± 0.12b	16.5 ± 0.41b	16.2 ± 1.37b	11.9 ± 0.16b
***P*-value**				
System	< 0.001	< 0.001	0.006	0.054
Time	0.505	< 0.001	0.409	0.306
Depth	< 0.001	< 0.001	< 0.001	< 0.001
System × Time	0.449	0.991	0.786	0.924
System × Depth	< 0.001	< 0.001	0.689	0.141
Time × Depth	0.143	< 0.001	0.1	0.282
System × Time x Depth	0.503	0.971	0.839	0.998

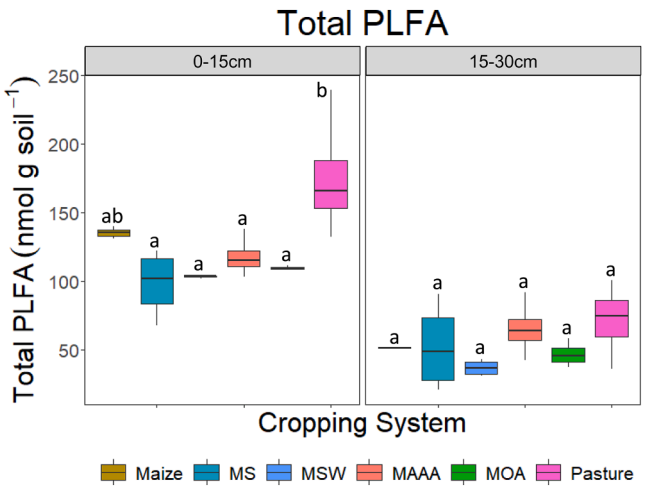

Fig. 4. Total PLFA content at 0–15 cm and 15–30 cm in WICST. Vertical lines indicate the standard errors. Maize; MS, maize and soybean; MSW, maize, soybean, and wheat; MAAA, maize, alfalfa, alfalfa, alfalfa; MOA, maize, oats/alfalfa, alfalfa; Pasture.

was also similar between Maize, MS, MSW, and MOA. Total PLFA and absolute abundance of microbial groups significantly also varied across depth, being significantly lower at 15–30 cm than at 0–15 cm.

^{18}O-DNA stable isotope probing was used to determine differences in growth rates and CUE across cropping systems and time. Microbial ^{18}O-CUE did not significantly differ between systems, only sampling time (Table 3, Time $P < 0.05$). CUE at mid-season was on average 46 % higher than late-season. Microbial growth rates (MGR) assessed via ^{18}O-H_20 incorporation significantly varied across systems (Fig. 5; System $P < 0.05$). For example, Pastures supported higher MGR than all other systems except Maize across both sampling times, with Pasture being significantly higher on average by 10–60 % at mid-season and 31–38 % at late-season. MGR values were also significantly lower at late-season than at mid-season by 77 %.

Microbial respiration, a component of microbial CUE, also significantly varied across systems, with Pasture being significantly higher by nearly 2x compared to all other systems across both sampling times (Table 3; System $P < 0.05$). PMC significantly varied across sampling time, with mid-season values being 25 % higher on average than late-season.

3.3. Microbial necromass contributions to long-term SOC stabilization

Total amino sugar content significantly varied across systems (Table 4; System $P < 0.05$), ranging from 750 to 1026 μg g^{-1}. Total amino sugar content did not significantly vary across time or depth. Glucosamine (GluN; fungal derived) content significantly varied across system, time, and depth, however depth effects differed across time (Table 4; Table S3; Time×Depth $P < 0.05$). For example, at mid-season sampling, GluN was significantly higher at 0–15 cm than 15–30 cm by 38 % but this effect was not observed at late-season sampling. Muramic acid (MurA; bacterial derived) content significantly varied across systems, but not time or depth (Table 4; System $P < 0.05$). Maize and MS were significantly higher than MSW by 37 %, and higher than all other systems by 7–16 %.

The ratio of GluN to MurA (GluN:MurA) did not significantly vary across systems, but varied across time and depth, however depth effects differed across time (Table 4; Table S3; Time×Depth $P < 0.05$). Similar to GluN, at mid-season sampling, GluN:MurA was significantly higher at 0–15 cm than 15–30 cm by 38 %, however this effect was not observed at late-season sampling.

Table 2
Total PLFA content, selected microbial group content, and PLFA ratios by cropping system and depth at WICST. Different letters indicate significant differences across cropping system or depth interval ($p < 0.05$). Maize; MS, maize and soybean; MSW, maize, soybean, and wheat; MAAA, maize, alfalfa, alfalfa, alfalfa; MOA, maize, oats/alfalfa, alfalfa; Pasture.

System	Total PLFA (ng g^{-1})	Actinomycetes (ng g^{-1})	AM Fungi (ng g^{-1})	Fungi (ng g^{-1})	Bacteria (ng g^{-1})	Gram Negative Bacteria (ng g^{-1})	Gram Positive Bacteria (ng g^{-1})	F:B	G+:G-
Maize	92.7 ± 10.46ab	9.49 ± 0.86a	2.91 ± 0.31a	10.33 ± 1.28a	51.3 ± 4.07ab	21.5 ± 1.78ab	20.3 ± 1.81ab	0.184 ± 0.009ab	1.001 ± 0.024ab
MS	75.5 ± 8.20a	8.32 ± 0.86a	2.79 ± 0.31a	7.64 ± 1.19a	42.0 ± 3.84a	18.0 ± 1.58a	16.8 ± 1.74a	0.176 ± 0.009a	0.976 ± 0.022ab
MSW	69.4 ± 8.81a	7.57 ± 0.90a	2.28 ± 0.31a	7.48 ± 1.19a	37.6 ± 4.07a	16.2 ± 1.68a	15.2 ± 1.81a	0.183 ± 0.009ab	1.019 ± 0.022a
MAAA	91.8 ± 8.20ab	9.22 ± 0.94a	3.51 ± 0.29a	10.73 ± 1.19a	49.9 ± 3.84ab	21.9 ± 1.58ab	17.5 ± 1.88a	0.206 ± 0.009bc	0.942 ± 0.024ab
MOA	79.0 ± 8.81a	9.39 ± 0.90a	2.65 ± 0.29a	8.38 ± 1.28a	43.1 ± 3.84a	18.5 ± 1.58a	17.7 ± 1.74a	0.183 ± 0.009ab	1.015 ± 0.021a
Pasture	123.7 ± 8.20b	13.24 ± 0.86b	4.84 ± 0.29b	15.88 ± 1.19b	61.2 ± 4.07b	27.4 ± 2.4b	24.1 ± 1.81b	0.221 ± 0.009c	0.917 ± 0.022b
Depth									
0–15 cm	123.3 ± 5.56a	12.98 ± 0.71a	4.80 ± 0.18a	15.41 ± 0.77a	65.6 ± 2.91a	29.5 ± 1.15a	24.8 ± 1.47a	0.225 ± 0.007a	0.862 ± 0.016a
15–30 cm	54.1 ± 5.56b	6.09 ± 0.69b	1.53 ± 0.17b	4.73 ± 0.74b	29.4 ± 2.81b	11.7 ± 1.12b	12.4 ± 1.44b	0.160 ± 0.007b	1.094 ± 0.015b
***P*-value**									
System	< 0.001	< 0.001	< 0.001	< 0.001	0.001	< 0.001	0.002	< 0.001	0.005
Depth	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
System×Depth	0.138	0.156	0.084	0.044	0.237	0.141	0.226	0.78	0.236

Table 3
Microbial CUE, MGR and Respiration (PMC) by cropping system and sampling time at WICST. Different letters indicate significant differences across cropping system or depth interval ($p < 0.05$). Maize; MS, maize and soybean; MSW, maize, soybean, and wheat; MAAA, maize, alfalfa, alfalfa, alfalfa; MOA, maize, oats/alfalfa, alfalfa; Pasture.

System	CUE	MGR (μg C g^{-1} soil hr^{-1})	Respiration (μg C g^{-1} soil hr^{-1})
Maize	0.58 ± 0.05	6.88 ± 0.65ab	106.8 ± 11.9a
MS	0.49 ± 0.05	4.71 ± 0.58a	83.2 ± 10.3ab
MSW	0.60 ± 0.05	4.99 ± 0.58a	72.4 ± 10.3b
MAAA	0.58 ± 0.04	5.84 ± 0.58a	93.0 ± 10.3ab
MOA	0.58 ± 0.05	5.18 ± 0.62a	73.0 ± 11.9ab
Pasture	0.46 ± 0.05	8.23 ± 0.62b	169.9 ± 11.9c
Sampling Time			
mid-season	0.65 ± 0.03b	7.63 ± 0.42b	110.7 ± 4.5b
late-season	0.45 ± 0.03a	4.31 ± 0.43a	88.8 ± 4.1a
***P*-value**			
System	0.067	< 0.001	< 0.001
Time	< 0.001	< 0.001	< 0.001
System:Time	0.438	0.84	0.777

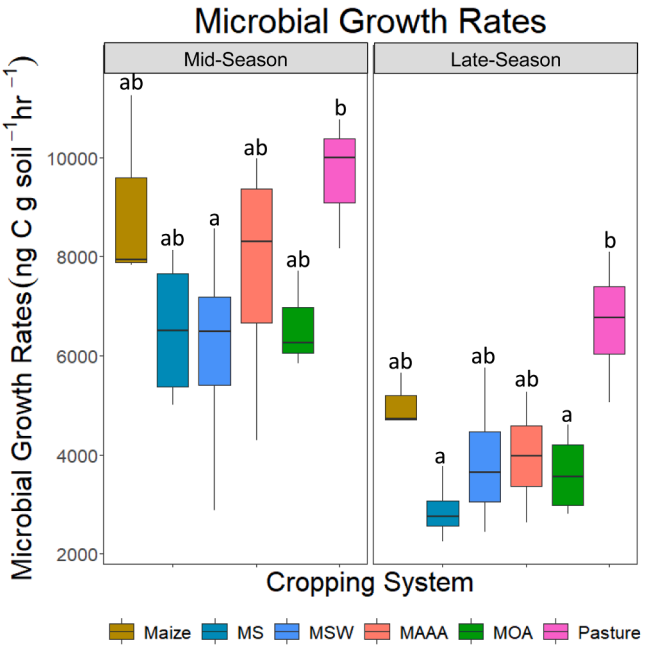

Fig. 5. Microbial growth rates at mid and late-season sampling at 0–15 cm in WICST. Vertical lines indicate the standard errors. Maize; MS, maize and soybean; MSW, maize, soybean, and wheat; MAAA, maize, alfalfa, alfalfa, alfalfa; MOA, maize, oats/alfalfa, alfalfa; Pasture.

3.4. Correlations among measured variables

Pearson's correlation analysis revealed several strong ($r > 0.75$) and moderate ($0.5 < r < 0.75$) correlations between measured parameters across times and soil depth (Figs. S1-S4). At 0–15 cm, MGR, PMC, total PLFA, and POM-C all had strong positive correlations to each other and strong negative correlations to CUE at mid-season. At late-season, all strong correlations to CUE observed at mid-season weakened, and strong positive correlations between MGR and PMC and PMC to POM-C persisted. POM C:N exhibited moderate negative correlations to PMC, POM-C, and MGR at mid-season, and moderate positive correlations to all amino sugars. At late-season, all moderate correlations between POM C:N and amino sugars weakened.

At 15–30 cm, POM-C exhibited moderate positive associations to total PLFA, F:B, and MAOM-C, and a negative moderate correlation to POM C:N at mid-season. MAOM-C maintained a moderate negative correlation to POM C:N, and moderate positive correlations to POM-C and almost all amino sugars across both sampling times.

4. Discussion

4.1. High-quality C inputs key to SOM maintenance and accrual

Our study reveals significant effects of cropping systems, sampling time, and depth on C contributions to SOM fractions, microbial community composition, and indicators of soil microbial function. As expected, Pasture supported SOM maintenance and build-up through high quality plant and animal C inputs, indicated by POM-C/C:N. In turn, high-quality POM supported greater biological function indicated by higher PMC and microbial growth/turnover rates. However, these measured responses significantly varied between sampling times. Significant system effects for these indicators, found at mid-season, were dampened at late-season, suggesting that microbial contributions to persistent SOM in both annual and perennial cropping systems are subject to seasonal dynamics and diminish from mid-season to late-season. Depth also significantly affected measured chemical and biological variables and their associations to MAOM-C, generally decreasing from 0 to 15 cm to 15–30 cm.

4.2. Cropping system differences most apparent at mid-season sampling

The findings of this study support the hypothesis (iii) that soil biological and chemical characteristics vary across the growing season. Assessments of microbial function are pivotal in a microbially-driven framework for SOM turnover and persistence; consideration as to the seasonal variation of these functions could improve larger conclusions about cropping system effects on SOM (Thompson et al., 2024; Wang et al., 2023; Q. Wang et al., 2021). In our study, significant decreases in microbial CUE, growth, and PMC, from mid-season to late-season, demonstrate the possibility of greater plant and climate-specific influences on soil biological characteristics at mid-season compared to late-season (Lange et al., 2024; Patra et al., 2021; Zhao et al., 2022). The chemical composition of POM also significantly varied across the growing season. For example, POM C:N significantly increased across systems at late-season, but only at 15–30 cm, possibly signaling high N mineralization at mid-season, which in turn led to lower organic N and higher C:N at late season (Walkup et al., 2020). Relatively strong associations among POM-C and POM C:N to microbial growth, CUE, and PMC in our study are consistent with previous findings suggesting that POM (i.e., litter) chemistry regulate microbial processes (Cheng et al., 2023; Witzgall et al., 2021). Interestingly, the strength of these associations decrease from mid to late-season sampling, indicative of significant temporal variation of these measurements in topsoil layers (Adingo et al., 2021; Sarkar et al., 2024). While our study did not show any interactive effects between sampling time and cropping system, previous studies have, complicating our understanding of SOM turnover and

Table 4
Concentration of amino sugars including glucosamine (GluN), muramic acid (MurA), total amino sugars, and GluN:MurA by cropping system, time, and depth at WICST. Different letters indicate significant differences across cropping system or depth interval ($p < 0.05$). Maize; MS, maize and soybean; MSW, maize, soybean, and wheat; MAAA, maize, alfalfa, alfalfa, alfalfa; MOA, maize, oats/alfalfa, alfalfa; Pasture.

System	GluN (ug g^{-1})	MurA (ug g^{-1})	GluN:MurA	Total Amino sugars (ug g^{-1})
Maize	657 ± 53.9	113.1 ± 8.16a	5.99 ± 0.28	1026 ± 87.8
MS	724 ± 53.9	113.2 ± 8.16a	6.06 ± 0.28	1026 ± 87.8
MSW	510 ± 55.9	82.6 ± 8.41b	6.25 ± 0.29	750 ± 90.5
MAAA	565 ± 51.7	97.6 ± 7.92ab	5.56 ± 0.28	821 ± 85.1
MOA	643 ± 51.7	105.3 ± 7.92ab	5.63 ± 0.28	897 ± 85.1
Pasture	728 ± 53.9	106.1 ± 7.92ab	6.46 ± 0.28	1002 ± 85.1
Time				
Mid-Season	699 ± 30.3a	102 ± 5.59	6.54 ± 0.213a	861 ± 51.2
Late-Season	577 ± 31.5b	104 ± 4.96	5.45 ± 0.196b	979 ± 58.2
Depth				
0–15 cm	694 ± 31.1a	98.9 ± 5.69	6.60 ± 0.216a	975 ± 59.3
15–30 cm	582 ± 30.7b	107.1 ± 4.87	5.39 ± 0.194b	866 ± 50.1
***P*-value**				
System	0.037	0.019	0.084	0.04
Time	0.009	0.842	< 0.001	0.213
Depth	0.016	0.156	< 0.001	0.18
System × Time	0.836	0.562	0.759	0.669
System × Depth	0.314	0.574	0.364	0.391
Time × Depth	0.007	0.947	< 0.001	0.069
System × Time x Depth	0.783	0.812	0.51	0.846

persistence, and furthering the argument for time-series sampling in future studies (Brown and Jones, 2024; Schnecker et al., 2023).

4.3. POM quantity and quality greatest under well-managed pasture systems

Our findings support hypothesis (i) that increased C input quantity and quality directly support POM accumulation in Pasture, which serves as substrate source for microbial metabolism. Decreasing POM C:N also clearly reflected the ecological gradient of higher quality plant C inputs created by more perennialized cropping systems with inclusion of low C:N plant and animal residues, specifically in the upper 15 cm of soil. Similar to POM C:N, more perennialized, forage-based systems with greater plant and animal C inputs tended to have greater POM-C, but only Pasture consistently promoted greater POM-C than all other systems, aligning with previous findings from WICST (Cates et al., 2016; Rui et al., 2022). Both the quantity and quality of POM, a direct product of plant inputs, can serve as a functional component for determining SOM persistence (Witzgall et al., 2021), and a direct substrate source that regulates microbial processes such as microbial growth and CUE (Cheng et al., 2023; Liang et al., 2017; Manzoni et al., 2012). However, our findings suggest that semi-perennial systems rotated with forage crops may not reliably enhance POM in corn-based systems, and their effect on soil microbial functions could be short-lived and rotation-dependent.

4.4. Microbial community activity highest under well-managed pasture systems

Pasture supported the greatest microbial respiration/PMC and microbial growth rates (assessed via incorporation of ^{18}O into DNA), supporting hypothesis (ii). Our results suggest that PMC and microbial growth rates are effective indicators of C sequestration across all cropping systems, supported by their strong and moderately strong associations to POM-C and POM C:N. Likewise, both POM-C and POM C:N may serve as effective indicators of microbial activity and abundance, however correlations consistently revealed that POM-C maintained the strongest associations to microbial metrics including MGR, PLFA, and necromass across all sampling times and depths. These results are consistent with previous studies that iterate how pastures promote soil health metrics related to C availability (Augarten et al., 2023; Cates et al., 2016; Diederich et al., 2019), but differ from previous work that determined that MOA system had significantly higher MBC than pasture (Rui et al., 2022), based on a different methodological approach (fumigation-extraction).

Microbial CUE assessed via a substrate independent ^{18}O-H_2O method produced confounding results that does not align with previous findings at WICST where ^{13}C- CUE was highest in Pasture (Rui et al., 2022), and does not support our initial hypothesis that CUE would be higher in systems with more energetically favorable C inputs (i.e., POM). CUE was lowest in pasture and had strong negative associations with microbial growth, PMC and POM-C at mid-season sampling and had weak to no associations at late-season sampling.

While many contributing factors may have led to these results, a parsimonious explanation lies in the methodological differences between ^{13}C and ^{18}O CUE approaches. In the ^{13}C substrate-based approach, the measurements required for calculating CUE (respiration and microbial biomass carbon; MBC) are specific only to the substrate that was added, that is the respiration and incorporation of ^{13}C substrate into microbial biomass, disregarding the total C substrate pool. For example, in Rui et al. (2022), ^{13}C respiration was lowest in Pasture, which accounted for higher CUE than all other systems. In contrast, the ^{18}O-H^2O approach measures total microbial respiration, alternatively described as PMC, which reflects the metabolism of all readily available soil C (Hurisso et al., 2016). Ultimately, high Pasture PMC drove down CUE in our study, compared to low Pasture ^{13}C respiration in Rui et al. (2022), which resulted in the highest CUE. These results further suggest that CUE data should be interpreted with caution, considering differences between methods and accounting for methodology-derived variations.

4.5. C input quantity and quality drive microbial abundance, composition, and contributions to persistent SOM

Our findings showed that microbial community abundance significantly varied across cropping systems, where pastures generally supported the highest PLFA abundance of all microbial groups and had the highest F:B. This aligns with previous research at WICST (Potter et al., 2022), as well as in other cropping system studies across the eastern and midwestern US which observed positive effects on the microbial community of both pasture systems and pastures integrated into grain and forage-based systems (Bansal et al., 2022; Walkup et al., 2020).

Interestingly, we found that Maize and MAAA were not significantly different from Pasture for total PLFA, bacteria, and gram-negative bacteria (Table 2).

Similarly, microbial necromass contributions and their associations to persistent SOM were significantly affected by cropping system, along with sampling time and depth. For example, at mid-season, 0–15 cm, primarily fungal derived necromass (GluN) was highest in Pasture, reflecting the active fungal community assessed by PLFA. However, GluN was highest in Maize in late-season, possibly indicating a seasonal shift in microbial community biomass and turnover. This suggests that microbial necromass, as represented by amino sugar biomarkers, reflects more stabilized residues from long-term soil processes but may still be subject to temporal fluctuations, so interpreting long-term impacts of cropping systems using amino sugar data should be done cautiously. GluN:MurA, an indicator of fungal:bacterial necromass, in Pasture, was significantly greater than all other systems at mid-season, reflecting the F:B of the active microbial community by PLFA. GluN:MurA had a moderate positive association to F:B at mid-season, 0–15 cm, supporting the idea that cropping systems which support greater fungal abundance would also support greater necromass contributions towards SOM (B. Wang et al., 2021). All amino sugars had stronger associations to MAOM-C at 15–30 cm than at 0–15 cm, suggesting that microbial, more so than plant contributions drive SOM accumulation at lower depths.

Both sampling time and depth affected associations between soil biological measurements and POM to MAOM-C, suggesting unique controls on SOM turnover across soil depths and time. MAOM-C had stronger associations to CUE, MGR, and PMC at mid-season than late season at 0–15 cm. While this aligns with our understanding of how biological processes may be affected by seasonal differences in climate and plant C inputs, it also limits our ability to interpret data related to MAOM-C formation and turnover. Our findings also suggest that POM has more direct associations to MAOM-C at 15–30 cm, possibly due to less POM-C being allocated to microbial respiration and maintenance.

4.6. Limitations and future directions

This study presents field observations that indicate strong seasonality of cropping system influence on soil microbial communities and processes that govern persistent SOM production. This study was limited to two sampling times in July and September, which does not fully capture the dynamic microbial processes that govern annual and even seasonal SOM turnover. Future studies would benefit from more frequent sampling to increase a greater time-resolved understanding of cropping system impacts on soil chemical and biological characteristics. This study was also performed on one sampling location and is limited in its capacity to relate observations beyond a regional scope. Future studies including replicate cropping systems across larger spatial scales would improve the scale of inference of our observations.

Lastly, we acknowledge the methodological limitations of ^{18}O-CUE (Geyer et al., 2019). In this approach, we applied a single regression model to determine the relationship between DNA and MBC concentration across all six systems. While this approach often utilized (Chernysheva et al., 2023; Gong et al., 2021; Joergensen et al., 2024), conducting individual linear regressions for each system may better account for system-specific abiotic and biotic differences in the DNA concentration/biomass relationship. Additionally, comparison of substrate-dependent and independent microbial CUE methods within these system-level studies would greatly benefit our interpretation of CUE data. Finally, concurrent in-situ ^{13}C tracing of plant and other C substrates would provide a comprehensive view of C fate into persistent SOM fractions and serve to leverage the strengths of lab and field-based approaches.

5. Conclusions

In summary, our results not only support that perennial pastures build SOM through plant inputs that enhance microbial composition and function, but also provide new insights into the temporal dynamics of microbial contributions to MAOM. While these effects are transient, increased rotational diversity and higher quality plant C inputs promote efficient microbial growth and improve POM substrate quality. These findings suggest that such practices could play a critical role in maintaining SOM and supporting ecosystem services, highlighting the importance of microbial communities in sustainable land management. Significant seasonal effects on POM C:N, PMC, MGR, and microbial necromass biomarkers highlight the need to consider seasonal variation when interpreting soil data. This finding emphasizes the temporal complexity of SOM dynamics, which has important implications for future studies that aim to better understand microbial mechanisms of SOM persistence and turnover. By reinforcing the idea that rotationally grazed pasture systems more effectively support POM-C compared to other cropping systems, our research contributes to a more nuanced understanding of how different system-level management strategies influence microbial processes and SOM stability. Ultimately, this work enhances our understanding of temporal and depth-dependent mechanisms of SOM persistence and offers valuable insights for developing more sustainable agricultural practices tailored to improving soil health.

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CRediT authorship contribution statement

Gregg R. Sanford: Writing – review & editing. **Matthew D. Ruark:** Writing – review & editing, Resources, Funding acquisition. **Yichao Rui:** Writing – review & editing, Conceptualization. **Tanner C. Judd:** Writing – original draft, Visualization, Investigation, Data curation. **Zachary B. Freedman:** Writing – review & editing, Supervision, Resources, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agee.2025.109769.

Data availability

Data will be made available on request.

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