Quantify the sources of MAOM by stoichiometry

Title: A stoichiometric approach to estimate sources of mineral-associated soil organic matter

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ABSTRACT

Mineral-associated soil organic matter (MAOM) is the largest, slowest-cycling pool of carbon (C) in the terrestrial biosphere. MAOM is primarily derived from plant and microbial sources, yet the relative contributions of these two sources to MAOM remain unresolved. Resolving this issue is essential for managing and modeling soil carbon responses to environmental change. Microbial biomarkers, particularly amino sugars, are the primary method used to estimate microbial versus plant contributions to MAOM, despite systematic biases associated with these estimates. There is a clear need for independent lines of evidence to help determine the relative importance of plant versus microbial contributions to MAOM. Here, we synthesized 288 datasets of C/N ratios for MAOM, particulate organic matter (POM), and microbial biomass across the soils of forests, grasslands and croplands. Microbial biomass is the source of microbial residues that form MAOM, whereas the POM pool is the direct precursor of plant residues in MAOM. We then used a stoichiometric approach – based on two-pool, isotope-mixing models – to estimate the proportional contribution of plant residue versus microbial sources to the MAOM pool. Depending on the assumptions underlying our approach, microbial inputs accounted for between 34%-47% of the MAOM pool, whereas plant residues contributed 53%-66%. Our results therefore challenge the existing hypothesis that microbial contributions are the dominant constituents of MAOM. We conclude that biogeochemical theory and models should account for multiple pathways of MAOM formation, and that multiple independent lines of evidence are required to resolve where and when plant versus microbial contributions are dominant in MAOM formation.

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Keywords: *soil carbon, mineral-associated organic matter, plant carbon, particulate organic matter, soil organic matter dynamics, meta-analysis*

INTRODUCTION

Soil organic matter (SOM) is Earth's largest actively cycling reservoir of carbon (C). Mineral-associated organic matter (MAOM) is the largest pool of SOM in Earth's mineral soils (>1500 Pg C), as well as the slowest-cycling, since physico-chemical interactions between SOM and the mineral matrix limit microbial access and decomposition (Kleber et al., 2021; Sokol et al., 2022b). MAOM is primarily composed of relatively simple biomolecules, which interact with soil minerals to form MAOM via two dominant pathways (Sokol et al., 2019). In the first pathway, plant inputs are assimilated by microorganisms and transformed into microbial cellular components; as microbial cells die and turnover, their residues interact with soil minerals to form 'microbial-derived' MAOM (Liang et al., 2017). In the second pathway, plant inputs directly interact with soil minerals without passing through microbial cells (i.e., 'plant-derived MAOM') – either in their intact form (e.g., direct sorption of simple sugars and amino acids from root exudates), or after partial decomposition of more complex plant compounds (e.g., lignin) by extracellular enzymes into simpler compounds (Sokol et al., 2019).

While both microbial and plant inputs form MAOM, their relative contributions remain unresolved, as do the environmental factors controlling their relative contributions (Angst et al., 2021; Whalen et al., 2022). Resolving these unknowns will inform (1) our basic understanding of the dominant controls on MAOM formation, and (2) how these controls are represented in biogeochemical models (Liang et al., 2017; Cotrufo & Lavallee, 2022; Sokol et al., 2022b). For instance, the formation of ‘microbial-derived MAOM’ centers microbial traits like growth rate and carbon-use efficiency (CUE) (Kallenbach et al., 2016; Hu et al., 2022a), whereas the formation of plant-derived MAOM may center traits like extracellular enzyme production, which can directly trade-off with growth rate and CUE (Malik et al., 2019; Sokol et al., 2022a).

Several approaches have been used to quantify plant versus microbial contributions to MAOM, each with their own strengths and shortcomings (Whalen et al., 2022). These approaches include: (i) microbial biomarker analysis (e.g., amino sugars, lipids) (Ludwig et al., 2015; Angst et al., 2021), (ii) ‘molecular fingerprinting’ approaches, that compare spectra or peaks of soil C to plant or microbial inputs (e.g., NMR or pyrolysis GC-MS) (Simpson et al., 2007; Grandy & Neff, 2008), and (iii) mathematical models (Klink et al., 2022). To date, the most common approach to quantify the contribution of microbial products to SOM are amino sugar microbial biomarkers (Appuhn & Joergensen, 2006; Liang et al., 2020). While amino sugars biomarkers have shed light on the relative contributions of microbial compounds to SOM and MAOM pools, the amino sugar approach also comes with several limitations (Joergensen, 2018; Liang et al., 2019; Whalen et al., 2022). Due to limitations in the amino sugar biomarker approach and other existing approaches, there is a critical need for additional, new approaches to independently and quantitatively determine plant versus microbial contributions to MAOM (Liang et al., 2020; Whalen et al., 2022). As each existing and new approach will likely bring its own unique set of shortcomings, multiple independent lines of evidence are required to develop the most robust estimates.

The stoichiometry of microorganisms and plants provides one such additional and independent source of data for evaluating the relative contributions of plant and microbial inputs to MAOM formation. SOM consistently has a larger C/N than microbial biomass C/N and a lower C/N than plant inputs because it contains a mixture of plant and microbial residues (Coonan et al., 2020). By extension, if the C/N ratio of MAOM is intermediate between microbial biomass and plant residues, MAOM then also contains some proportion of the two. Comparing C/N ratios of these soil C pools may yield important and independent insights on the contributions of plant and microbial residues to MAOM pools. To date, few if any studies have comprehensively compared the C/N ratios of microbial biomass, plant residues and MAOM across a broad range of environmental conditions, nor used their stoichiometry to estimate plant residue versus microbial contributions to MAOM.

Here, we put forward a new line of evidence to estimate the contributions of plant and microbial residues to MAOM based on their stoichiometry. Plant residues are primarily incorporated into MAOM via the particulate organic matter (POM) pool (Coonan et al., 2020; Witzgall et al., 2021; Cotrufo & Lavallee, 2022), which consists largely of partially decomposed plant compounds (Guigue et al., 2021). Thus, the C/N ratio of POM can be used to estimate plant residue inputs to MAOM. Central to the isotope mixing approach is that the values of the source materials, such as POM and microbial biomass, differ to the mixture (i.e., MAOM) and to one another, bracketing the mixture. These differences generally hold for POM, MAOM and microbial biomass (Cleveland & Liptzin, 2007; Xu et al., 2013; Cotrufo et al., 2019; Amorim et al., 2022). Although the stoichiometry of these pools is commonly expressed as C/N ratios, we used the fractional abundance of N (i.e., N/(C+N)) to estimate their relative contributions. This approach avoids the spurious inferences that can arise from using ratios (e.g., Jasieński & Bazzaz, 1999), and instead adapts the approach used in enriched isotope mixing-models in ecology to identify the contributions of sources to a mixture. Specifically, as with enriched stable isotopic approaches, ratios cannot be used in mixing equations because the difference between the light and heavy isotope in the denominator is no longer essentially a constant – which it is with natural abundance approaches – meaning that ratios must be converted to fractional abundances for mixing equations (Fry, 2006). We therefore propose that the fractional abundance of N in microbial biomass and POM can be used as end-members in a mixing model to differentiate their contributions to MAOM, providing a novel line of evidence to help evaluate the relative contributions of plant- and microbial-derived materials to MAOM.

We collected 288 published sets of data that report C/N ratios of microbial biomass, POM and MAOM. These data mainly cover three ecosystems (forests, grasslands, croplands) and different soil depths. We expected that MAOM would have higher C/N values than microbial biomass and lower C/N values than POM, because MAOM typically contains a mix of both plant and microbial residues. Subsequently, we used the ecosystem-specific combination of the fractional abundance of N, i.e., [N/(C+N)] of POM, microbial biomass, and MAOM to assess the relative plant and microbial residue contributions to MAOM.

METHODS

Data collection and extraction

We collected data from peer-reviewed articles published before August 2022 on Google Scholar and Web of Science, which simultaneously reported the C/N ratios (or carbon and nitrogen contents) of three soil organic matter pools: 1) microbial biomass, 2) POM (or light fraction), and 3) MAOM (or heavy fraction). MAOM and POM pools were defined both by size fractionation and density fractionation, as has been done in a previous meta-analysis (Georgiou et al., 2022). For each study in our dataset, we also extracted corresponding data on climate (mean annual temperature (MAT), mean annual precipitation (MAP)) and soil properties (clay content, pH, soil organic carbon, total N, soil C/N). The missing data of climate and soil properties were supplemented by searching from global GIS datasets by latitude and longitude. These variables were selected to represent the broadest variation in environmental characteristics following van den Hoogen et al. (2019), and to capture the environmental drivers that have been hypothesized to influence microbial growth and activity in soil (Crowther et al., 2019).

Papers had to meet the following criteria to be included in our dataset: microbial biomass carbon (MBC) and nitrogen (MBN) were simultaneously measured by the chloroform fumigation extraction method. This is because separate measurements (e.g., MBC as measured by fumigation-incubation method and MBN by steam distillation for mineral N) of the two indexes would increase the uncertainty of microbial C/N. To keep consistency among all studies with various fractionation methods, multiple sub-POM fractions, e.g., fine POM, coarse POM, free POM, or occluded POM in some studies, were unified to obtain an overall C/N of POM by dividing the combined total C content of these fractions by their total N content.

In total, 294 groups of C/N ratios (one group included one C/N ratio of microbial biomass, POM, and MAOM, respectively) were collected from 36 peer-reviewed articles. Improbably low C/N values – based on empirical and theoretical knowledge – were removed (i.e., one group of POM C/N that was 1.9, and five groups of Microbial C/N that were < 2), resulting in 288 groups of C/N ratios from 36 peer-reviewed articles. All C/N data of POM, microbial biomass, and MAOM in this research are available at https://doi.org/10.5281/zenodo.10147884.

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Calculations and statistical analysis

A paired sample *t*-test was used to test the pairwise differences in C/N ratios between microbial biomass and MAOM, and between POM and MAOM, respectively. We used the combination of the fractional abundance of N of POM and microbial biomass to assess the relative contributions of plant and microbial residues to MAOM (Figure 1). We assumed that microbial biomass C/N ratio was similar to microbial necromass C/N, so the fractional abundance of N was the same between the two pools. The fractional abundance of N, i.e., [N/(C+N)], in microbe, POM, and MAOM pools was calculated based on their corresponding C/N ratios as following:

$$[N/(C+N)] = 1/(1+C/N) \qquad (1)$$

We used the fractional abundance of N in the two-end-member model to differentiate the contributions of microbial and plant residues to MAOM following:

$$f \times [N/(C+N)]_{\mathrm{Microbe}} + (1-f) \times [N/(C+N)]_{\mathrm{POM}} = [N/(C+N)]_{\mathrm{MAOM}} \qquad (2)$$

Where f is the proportion in MAOM that is derived from microbial residues, and thus 1-f is the fraction in MAOM originating from plant residues (i.e., POM). $[N/(C+N)]_{\mathrm{Microbe}}$ is the fractional abundance of N in microbial biomass. $[N/(C+N)]_{\mathrm{POM}}$ is the fractional abundance of N in POM. $[N/(C+N)]_{\mathrm{MAOM}}$ represents the fractional abundance of N in MAOM.

Therefore, the proportion of microbial residue to MAOM (f) can be estimated as:

$$f = \frac{[N/(C+N)]_{\mathrm{MAOM}} - [N/(C+N)]_{\mathrm{POM}}}{[N/(C+N)]_{\mathrm{Microbe}} - [N/(C+N)]_{\mathrm{POM}}} \qquad (3)$$

When $[N/(C+N)]_{\mathrm{MAOM}} > [N/(C+N)]_{\mathrm{Microbe}}$ (i.e., $C/N_{\mathrm{MAOM}} < C/N_{\mathrm{Microbe}}$), the values of f are larger than 1; when $[N/(C+N)]_{\mathrm{MAOM}} < [N/(C+N)]_{\mathrm{POM}}$ (i.e., $C/N_{\mathrm{MAOM}} > C/N_{\mathrm{POM}}$), f is smaller than 0. Within our collected 288 groups of data, 70 groups had C/N$_{\text{MAOM}}$ < C/N$_{\text{Microbe}}$, and 39 groups had C/N$_{\text{MAOM}}$ > C/N$_{\text{POM}}$. C/N$_{\text{MAOM}}$ < C/N$_{\text{Microbe}}$ may partly be attributed to MAOM containing some proportion of mineral N besides organic matter, e.g., the adsorption of ammonium on soil minerals (Mortland, 1959; Adams Jr. & Stevenson, 1964). However, according to Bimüller et al. (2014), the proportion of mineral N to total N in silt or clay sized MAOM was only 0.6~2.5%, thereby not substantially changing C/N ratios of MAOM. Accordingly, we speculated that C/N$_{\text{MAOM}}$ < C/N$_{\text{Microbe}}$ indicated that 100% of MAOM is from microbial residues and C/N$_{\text{MAOM}}$ > C/N$_{\text{POM}}$ indicates 100% of MAOM is derived from plant residues and nil derived from microbial residues, and the difference resulted from the measurement error of C/N. Therefore, these abnormal f-values were replaced by 1 and 0, respectively. We analyze and present the data both excluding and including these abnormal points.

The C/N ratios of organic matter pools and the proportion of microbial residues in MAOM were compared among grasslands, forests, and croplands using one-way ANOVA. As the sampled soil depth differed between experiments, the depth was expressed as the arithmetic mean of the upper and lower boundaries of each layer. Soil arithmetic mean depths were divided into two categories: < 20 cm as topsoil and ≥ 20 cm as subsoil; we then compared the average proportion of microbial residues in MAOM between the two depth categories using one-way ANOVA.

Regression analysis was performed to look at correlations among the C/N ratios of MAOM and microorganisms, and between MAOM and POM. In addition, random forest analysis was used to quantify the relative explanatory power of climate (MAT and MAP) and soil properties (clay content, pH, soil organic carbon, total N, soil C/N) on the microbial contribution to MAOM. All the paired sample *t*-tests, regression analyses and ANOVA analyses were conducted in SPSS 25.0 (SPSS Inc., Chicago, IL, USA). Random forest analysis was performed using the R “randomForest” package and the “rfPermute” package in the R Statistical Environment (Version 4.1.0, R Core Team).

Sensitivity test under scenarios for contributions of plant DOM to plant-derived MAOM

Root exudation and DOM from leaf litter leachate are also potential contributors to MAOM (Cotrufo & Lavallee, 2022), thus plant DOM may affect the estimation of our two-end-member model. If the C/N ratio of plant DOM is close to that of POM, the incorporation of DOM in the above two-end-member model does not substantially change the contribution of microbial residues to MAOM. However, some N-poor compounds (e.g., carbohydrates or aromatic acids) also likely contribute to MAOM by sorption with reactive mineral phases (Kramer et al., 2012; Whalen et al., 2022). According to Qualls and Haines (1991), C/N ratios of N-poor hydrophobic acids in DOM across various soil horizons and in streams ranged from 34 to 73. In this study, we assumed a C/N ratio of 50 for these N-poor components in DOM (the fractional abundance of N thereby 1/51), to test the sensitivity of our approach to the inclusion of plant DOM in MAOM. Unfortunately, no studies reported the proportion of DOM in plant-derived MAOM (Whalen et al., 2022). We assumed two scenarios, where the proportion of plant DOM contributions to plant-derived MAOM were either low (i.e., 10%) or high (i.e., 50%). We then adjusted the fractional abundance of N of plant-derived organic matter in these scenarios as follows:

$$\text{Scenario 1:}\quad [N/(C+N)]_{\mathrm{POM+DOM}} = [N/(C+N)]_{\mathrm{POM}} \times 90\% + [N/(C+N)]_{\mathrm{DOM}} \times 10\% \qquad (4)$$

$$\text{Scenario 2:}\quad [N/(C+N)]_{\mathrm{POM+DOM}} = [N/(C+N)]_{\mathrm{POM}} \times 50\% + [N/(C+N)]_{\mathrm{DOM}} \times 50\% \qquad (5)$$

Where $[N/(C+N)]_{DOM}$ is assumed to be 1/51. Then, we used the new combined $[N/(C+N)]_{POM+DOM}$ instead of $[N/(C+N)]_{POM}$ to estimate the microbial contribution to MAOM using equation (1).

Sensitivity test under scenarios for proportions of microbial residues in POM

Although POM is primarily composed of partially decomposed plant fragments (Golchin et al., 1994; Lavallee et al., 2020; Guigue et al., 2021), isotopic evidence suggests that some microbial necromass exists as POM (Wang et al., 2020), such as larger fungal fragments (Lavallee et al., 2020). Similarly, small amounts of microbial-derived amino sugars have been measured in POM (Turrión et al., 2002; Griepentrog et al., 2014). Because our approach uses POM to represent the contribution of plant residue inputs to MAOM, microbial residues in the POM fraction would decrease the C/N ratio and increase the fractional abundance of N, and may lead to an overestimation of the plant contribution to MAOM (an underestimation of the microbial contribution).

To assess the possible impact of this uncertainty, we estimated the proportion of microbial residue in POM, based on the amino sugar data in Griepentrog et al. (2014) and using the conversion method in Wang et al. (2021). These results suggest that the total fungal and bacterial necromass C accounted for 10% in free light fraction C and 15% in occluded light fraction C (light fraction is equivalent to POM, Table S1). Based on these results, we ran a simulation under different scenarios of microbial residue proportions in POM to evaluate how sensitive our assumptions were to the possibility that POM contains some microbial biomass and/or necromass. We assumed: 1) that if the proportion of microbial residues in POM is f_1, then the proportion of plant residues in POM is $1 - f_1$; and 2) that if the proportion of microbial residues in MAOM is f_2, then the proportion of plant residues in MAOM is 1 - f_2. Accordingly, the relationships between the fractional abundances of N in microbial biomass, POM and MAOM can be described by the following equations:

$$f_1 \times [N/(C+N)]_{\text{Microbe}} + (1-f_1) \times [N/(C+N)]_{\text{Plant}} = [N/(C+N)]_{\text{POM}} \tag{6}$$

$$f_2 \times [N/(C+N)]_{\text{Microbe}} + (1-f_2) \times [N/(C+N)]_{\text{Plant}} = [N/(C+N)]_{\text{MAOM}} \tag{7}$$

Where $[N/(C+N)]_{\text{Plant}}$ represents the fractional abundance of N in plant residue in POM and MAOM. Combining equations (6) and (7), we arrived at the following equation:

$$f_2 = \frac{[N/(C+N)]_{\text{MAOM}} - [N/(C+N)]_{\text{Microbe}}}{[N/(C+N)]_{\text{POM}} - [N/(C+N)]_{\text{Microbe}}} \times f_1 + \frac{[N/(C+N)]_{\text{POM}} - [N/(C+N)]_{\text{MAOM}}}{[N/(C+N)]_{\text{POM}} - [N/(C+N)]_{\text{Microbe}}} \tag{8}$$

We assumed two scenarios of when f_1 was 10% and 15%, as described above. Finally, we can obtain the proportion of microbial residues in MAOM (f_2) under the two scenarios, based on the N/(C+N) values of MAOM, POM, and microbe pools for a given soil.

RESULTS

C/N ratios of organic matter pools

The mean C/N ratio of MAOM was higher than that of microbial biomass in forests ($p < 0.001$), croplands ($p < 0.05$), and grasslands ($p = 0.11$), as well as across all ecosystem types ($p < 0.001$) (Figure 2). The C/N ratio of MAOM was lower than that of POM in all the three types of ecosystems (all $p < 0.001$) (Figure 2). The fact that the C/N ratio of MAOM fell between the C/N ratio of POM and microbial biomass (Figure 2) supports theoretical expectations that microbial and plant residues are both contributors to MAOM.

POM had a larger mean C/N ratio in forests (24.4) than in grasslands (19.2) and croplands (15.5)

($p < 0.001$, Figure 2). MAOM C/N was also larger in forests (13.8) than grasslands (12.4) and croplands (11.0) ($p < 0.001$). The mean C/N ratios of microbial biomass were, by contrast, approximately the same across forests, grasslands, and croplands ($p = 0.30$). Despite the differences of the mean C/N ratios of the SOM fractions and similarities microbial biomass among the three ecosystem types, the C/N ratios varied markedly within each ecosystem type. Overall, MAOM fractions had a narrower range (from 7.1 to 41.5) than those of POM (7.9 to 104.6) and microbial biomass (from 2.1 to 37.1).

MAOM C/N was positively associated with microbial biomass C/N in forests and grasslands ($p < 0.01$, Figure 3b, and 3c), as well as across all ecosystem types ($p < 0.05$, Figure 3a), but not in croplands (Figure 3d). The slope of the regression line between MAOM and microbial biomass C/N was steeper in forests (0.29) than in grasslands (0.14) and was also steeper than the slope across the entire data set (0.09). MAOM C/N was positively associated with POM C/N across all ecosystem types ($p < 0.001$, Figure 3a), within forests ($p < 0.001$, Figure 3b) and croplands ($p < 0.05$, Figure 3d), but not within grasslands (Figure 3c). The slope of the regression line between MAOM and POM C/N was steeper in forests (0.15) and across the entire data (0.12) than in croplands (0.05).

Microbial contributions to MAOM depend on ecosystem and soil depth

Stoichiometric analysis based on fractional abundances estimated that, on average, microbial inputs accounted for ~34% of contributions to MAOM across all environmental contexts (Figure 4a), whereas plant residue inputs (via POM) accounted for ~66%. The microbial contribution to MAOM was higher in forests (38%) and croplands (36%) than in grasslands (27%) ($p = 0.07$, Figure 4a). Across all ecosystem types, the microbial contribution to MAOM increased by 1.3 times from 33%

in topsoil (< 20 cm) to 42% in subsoil (≥ 20 cm) ($p = 0.09$, Figure 4b). Unfortunately, we did not have enough data to estimate the effect sizes of causal variables that might be generating the differences between soil depths and ecosystems. We instead used random forest analysis to look at which variables explained the most variation in the estimated contributions to MAOM, which suggested that soil clay content was the most important factor influencing the microbial contribution to MAOM (Figure 5). Microbial contribution to MAOM positively correlated with soil clay content ($p < 0.001$, Figure S1a), suggesting that the importance of microbial contributions to MAOM may be particularly pronounced in clay and loamy soils.

We observed a larger microbial contribution to MAOM on samples isolated via density fractionation than via size fractionation, though the general patterns observed (e.g., microbial contribution less than 50%, and lower microbial contribution in grassland than other ecosystem types) were consistent between both methods (Figure S2). Density fractionation is generally considered to be more effective at isolating the mostly plant-derived, particulate organic matter fraction (Leuthold et al., 2023), which may explain why this method was associated with more microbial-derived MAOM (Figure S2). Since there were more samples in our dataset isolated via density fractionation versus size fractionation (n=99 vs. n=80), this may have dampened the magnitude of effect we observed in terms of the overall plant contributions to MAOM.

Sensitivity analysis

In addition to POM, another potentially important source of plant C to the MAOM pool is DOM. We used two illustrative scenarios to estimate how N-poor DOM (assuming a C/N ratio of 50) inputs may affect our model results – 10% and 50% contributions of DOM to the plant-derived MAOM pool (see Methods). When the DOM contribution increases, the N fractional abundance of the combined POM+DOM pools decreases (i.e., a C/N increase) (Eq. 4 and Eq. 5), which then leads to estimates from the mixing models of increased microbial contributions to MAOM (Eq. 3). When assuming the proportion of DOM was 10%, the average microbial contributions were 35% (Figure S3a) – which is relatively close to our estimate when DOM was excluded (i.e., ~34%, Figure 4a). When assuming the proportion of DOM was 50%, the average microbial contribution increased to 47% (Figure S3c) – still less than 50% of the total pool. The microbial contribution to MAOM was higher in subsoil than in topsoil, regardless of whether the DOM contribution to plant-derived MAOM was 10% (Figure S3b) or 50% (Figure S3d).

Although POM is primarily composed of plant residues, it may contain some microbial residues (Guigue et al., 2021). To reveal the uncertainty associated with microbial contributions via POM, we modeled the two scenarios of 10% and 15% microbial residues in POM, based on the data in Griepentrog et al. (2014), as shown in Table S1. The average microbial contributions to MAOM increased from 34% to 41% and 44% under the two scenarios, respectively (Figure S4a,c). The comparisons of microbial contributions among different ecosystems and soil depths (Figure S4) and its relationship with climate and soil properties (Figure S5), did not substantially change.

Finally, in our dataset, there were dozens of datapoints where $C/N_{MAOM} < C/N_{Microbe}$, or $C/N_{MAOM} > C/N_{POM}$, which – using the mixing models – then led to estimated microbial contributions to MAOM of 100% and 0, respectively (see Methods). Instead of eliminating these datapoints from our analysis, we ran our analyses both without these datapoints (i.e., model results described above (Figure 4)), as well as with these abnormal data points (Figure S6), to see how they affected our results. When they were included, the average microbial contribution increased from

34% to 45%, i.e., still lower than the plant contributions (Figures 4a and S6a). The average microbial contribution increased from 38% to 43% in forests, from 27% to 46% in grasslands, and from 36% to 46% in croplands (Figures 4a and S6a). The higher microbial contribution to MAOM in subsoil than in topsoil and the relative explanatory power of climate and soil factors for the microbial contributions to MAOM did not change (Figures S6b and S7).

DISCUSSION

There is an increasingly popular view that microbial residues strongly dominate MAOM pools (Bradford et al., 2013; Cotrufo et al., 2013; Kögel-Knabner, 2017; Oldfield et al., 2018; Creamer et al., 2019; Buckeridge et al., 2020; Liang et al., 2020; Cotrufo & Lavallee, 2022; See et al., 2022). This view is built both on qualitative and semi-quantitative sources of evidence, such as imaging techniques that show microbial necromass-mineral associations (e.g., scanning electron microscopy; Miltner et al., 2012), as well as more directly quantitative approaches, such as the upscaling of microbial biomarkers (e.g., amino sugars) (Liang et al., 2019; Wang et al., 2021), 'molecular fingerprinting' approaches (e.g., ^{13}C-NMR or py-GC-MS) (Lehmann et al., 2007; Grandy & Neff, 2008; Lehmann et al., 2008; Solomon et al., 2012), and mathematical models (e.g., Bayesian Inference isotopic mixing model based on ^{13}C and ^{15}N natural abundance) (Klink et al., 2022). Yet, caveats in these existing approaches introduce uncertainty into quantitative estimates of microbial versus plant contributions to MAOM. This has precipitated calls for new, independent lines of evidence (Whalen et al., 2022). Here, to address that call, we conducted a meta-analysis of C/N ratios of MAOM, microbial biomass, and POM across different environmental contexts, and used a novel stoichiometric approach (via the fractional abundance of N) to estimate the contributions of plant residues (via the POM pool) and microbial residues to MAOM. Below, we first discuss the results of the meta-analysis, followed by the results of our stoichiometric approach.

Our meta-analysis showed that MAOM C/N ratios are greater than microbial C/N ratios and lower than POM C/N ratios within each of the three ecosystem types, as well as across all ecosystem types (Figure 2). These findings suggest that MAOM contains both microbial and plant residues, in agreement with prior studies. For example, a meta-analysis based on 74 studies across 29 countries showed that sand-sized fractions (equivalent of POM) have larger C/N ratios (median of 16.7) than silt-sized (median of 13.1) and clay-sized MAOM fractions (median of 9.6) (Amorim et al., 2022). This trend was also observed in a forest and a grassland soil (Ding et al., 2014) and two cropland soils including upland and paddy (Ding et al., 2018). An analysis based on 9,415 data points of European forest and grassland soils reported that the C/N ratio of MAOM (13 ± 5) was lower and less variable than that of POM (22 ± 15) (Cotrufo et al., 2019). Moreover, we found a close link between MAOM and POM across the entire dataset, especially in forest and cropland soils (Figure 3). Similar close relationships between C/N ratios of POM and MAOM, as well as for δ^{13}C, C–H (aliphatic)/C=O, and C=C (aromatic)/C=O), were observed across 156 soils spanning diverse ecosystems (tundra to tropics) in North America (Yu et al., 2022). Based on these relationships, they concluded that POM and MAOM are coupled, and that plant residues contribute substantially to the MAOM pool.

Overall, the results from our stoichiometric approach suggest that microbial inputs form less than 50% of MAOM across different ecosystem contexts. The overall mean microbial contribution was 34%-47% (Figures 4 and S3c), whereas the overall mean plant residue contribution to MAOM was 53%-66%. Our results align with other studies, which have also suggested that microbial inputs may not be the dominant constituent of the MAOM. For instance, in a synthesis of microbial biomarker studies, Angst et al. (2021) estimated an average contribution of microbial necromass to MAOM (“silt and clay fractions” in their study) of 39%, based on 60 groups of amino sugars data. Similarly, based on amino sugar data, microbial contributions to MAOM were 21%-30%, in an organic grain crop-cover crop rotation field in the USA (Zhang et al., 2022), and 13%-19% in subtropical forest and cropland in the southwestern karst region of China (Hu et al., 2022b). Chemical composition-based approaches have also suggested microbial inputs may not be dominant. For example, a study using pyrolysis-field ionization mass spectrometry, which used lipid and carbohydrates as biomarkers in four arable soils of widely differing properties, showed a 1:1 contribution for microbial and plant compounds to MAOM (Ludwig et al., 2015). A study using ^{13}C-NMR on an acidic forest soil concluded that microbial contributions to total SOM were less than 5% (Simpson et al., 2007). As emphasized in a recent review, the estimated proportions of MAOM that are microbial versus plant derived are method- and context-dependent (see Whalen et al. (2022) for a discussion on how different methods may influence these estimates).

We found that the contribution of microbial residues to MAOM increased with soil depth (Figure 4b). This is consistent with previous studies showing that the contribution of amino sugars to the SOM pool was greater in subsoil (Liang & Balser, 2008; Angst et al., 2018). These patterns were suggested to be caused by the different distributions across soil depths of plant and microbial residues. Kaiser et al. (2004) observed that the concentration of plant derived DOC (e.g., lignin-derived phenols) decreased with depth while that of microbial derived DOC (e.g., amino sugars) increased or remained constant. Consistent with this observation, we found a larger proportion of plant residues (indicated by POC in total SOC) in topsoil than in subsoil ($p < 0.001$, Figure S8).

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Kaiser et al. (2004) explained that the decrease in lignin-derived phenols was due to their strong affinity with Al and Fe oxides-hydroxides (Kaiser et al., 2002), and that hydrophobic DOC that was preferentially retained when transported through the mineral soil contained few amino sugars (Kaiser et al., 2001). Another explanation could be that the major dissolved molecules from plants are coming from the soil surface; in contrast, the microbial transformations are “similar” in top- and subsoil.

Among soil properties, texture was the most important factor influencing the relative contribution of microbial residues to MAOM (Figure 5), which increased with increasing clay content (Figure S1a). These results suggest that clay and loamy soils generally have larger microbial contributions to MAOM than sandy soils. This observation is supported by Yu et al. (2022) who found that the difference between C/N ratios of POM and MAOM increased with silt and clay contents (the larger the difference, the smaller the plant contribution to MAOM, and thereby the larger the microbial contribution). Similarly, the abundance of amino sugars in global grassland soils increases with clay contents (Ma et al., 2018). Compared to sand and silt particles, clay particles have much larger negative charged mineral surfaces and stronger aggregation (Ding et al., 2014; Ding et al., 2018), causing a stronger affinity for microbe-derived molecules (e.g., amino acids and amino sugars, containing the positively charged -NH_2 groups). These properties can protect the molecules against re-utilization by other microbes or higher trophic groups (Elliott et al., 1980; Ding et al., 2015; Islam et al., 2022) and enzymatic attacks (Baldock & Skjemstad, 2000). Accordingly, Grandy and Neff (2008) reported that microbially-derived compounds (e.g., N-containing compounds, lipids, waxes, aliphatics, and carbohydrates) increase with decreasing soil particle size. In addition, soils with high clay contents commonly have higher nutrient contents (Ristori, 1979), which favor the growth and turnover of microorganisms, as well as the formation of microbial residues (Creamer et al., 2016).

Notably, the contribution of microbial residues to MAOM were similar between forests and croplands, but they were higher than that in grasslands (*p* = 0.07, Figure 4a). These results likely reflect that within our dataset, clay content in grasslands was lower than in croplands and forests (Figure S1b). Our results are in line with Hu et al. (2022b) who observed similar microbial contribution to MAOM C in forest and cropland soils for both bacteria and fungi. In contrast, Angst et al. (2021) found that grasslands favor microbial necromass accumulation in MAOM relative to croplands or forests, however their grassland data points were very limited (n = 2). Importantly, the contribution of microbial residues to MAOM does not mirror its contribution to bulk SOM (Angst et al., 2021). Two recent meta-analysis studies, both of which used the amino sugars biomarker approach, found that the proportion of microbial necromass C in bulk SOC was lower in forests than grasslands and croplands (Liang et al., 2019; Wang et al., 2021). This could be due to the greater dominance of POM (mainly plant residues) in forest SOM pools (Figure S8), a result consistent with a recent multi-site analysis (Cotrufo et al., 2019).

There are important limitations to our method, which should be addressed by future studies to help build upon and refine our approach. First, our approach does not account for the possible impact of microbial death pathways on the chemical composition of microbial necromass (Camenzind et al., 2023). Once data becomes available on the impact of microbial death pathways on necromass chemistry, it should be used to fine-tune our approach. Second, other sources of organic input may be important for MAOM formation in addition to POM and microbial body residues, such as plant-derived DOM, amendments in croplands, or microbial extracellular products. To date, minimal data exists quantifying the role of these inputs on MAOM formation. Moreover, there is limited data for what proportion of POM may be derived from microbial inputs (Lavallee et al., 2020). It will be critical for future empirical efforts to determine if other sources of C input may influence the relative contributions of plant versus microbial inputs in different ecosystem contexts, to better constrain the proportion of microbial residues in the POM pool, as well as more directly ascertain how plant DOM and microbial extracellular residues contribute to the MAOM pool (Whalen et al., 2022). Third, our uncertainty estimates did not include measurement errors of C/N ratios, as data errors were not always reported. We encourage future studies to clearly report errors, make data openly available, and to use statistical approaches (such as Bayesian mixing models) that carry such errors forward. Last, few if any datasets exist which use multiple approaches – on a common set of soil samples – to measure plant versus microbial contributions to MAOM. As all current methods come with their own unique limitations, it will be critical to design studies that employ a suite of complementary approaches (such as different biomarkers, isotopic data, C/N ratios, chemical composition data, etc.) to develop the most robust estimates of plant versus microbial contributions to MAOM.

CONCLUSIONS

Our global comparison of the C/N ratios of microbial biomass, POM, and MAOM in forests, grasslands, and croplands demonstrated that MAOM C/N ratios are intermediate between those of POM and microbial biomass. By leveraging these 288 datasets of C/N ratios and using a novel stoichiometric approach (via the fractional abundance of N) to estimate the proportional plant and microbial contributions to the MAOM pool, we found that plant residue contributions to the MAOM pool (53%-66%) exceeded microbial contributions (34%-47%) across ecosystems. These results held when addressing key potential caveats, such as the role of a third source of input, the role of plant DOM in supplying the MAOM pool, and when including abnormal datapoints that we had removed from our primary analysis. While our results underscore that microbial residues are important constituents of MAOM, especially in deeper soils and in high clay soils, they challenge the increasingly popular view that microbial contributions are the dominant component of MAOM. Our results also underscore the need for other independent lines of evidence that quantitatively differentiate the role of plant versus microbial contributions to the MAOM pool. While there are limitations inherent to each method, multiple lines of evidence will be critical to determine the relative importance of plant versus microbial inputs. Such understanding is needed to advance a more robust theoretical knowledge of SOM dynamics, and for projecting their response to a changing climate.

AUTHOR CONTRIBUTIONS

Yi Chang: Formal analysis, Investigation, Validation, Visualization, Writing – original draft. **Noah W. Sokol:** Methodology, Validation, Visualization, Writing – original draft, Writing - Review & Editing. **Kees Jan van Groenigen:** Validation, Writing - Review & Editing. **Mark. A. Bradford:** Methodology, Validation, Writing - Review & Editing. **Dechang Ji:** Formal analysis. **Thomas W. Crowther:** Writing - Review & Editing**. Chao Liang:** Writing - Review & Editing. **Yiqi Luo:** Writing - Review & Editing. **Yakov Kuzyakov:** Visualization, Writing - Review & Editing. **Jingkuan Wang:** Project administration, Resources, Supervision. **Fan Ding:** Conceptualization, Methodology, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing - Review & Editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Supporting Information and Zenodo at https://doi.org/10.5281/zenodo.10147884.

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SUPPLEMENTARY INFORMATION

Supplementary data to this article can be found online.

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Figure legends

FIGURE 1 Schematic diagram to illustrate the two-end-member mixing model to estimate the contribution of microbial and plant residues to mineral-associated organic matter (MAOM).

Quantify the sources of MAOM by stoichiometry
Fractional abundance of N [N/(C+N)] in particulate organic matter (POM) and microbial biomass were used as end-members in a mixing model to estimate plant versus microbial contributions to MAOM. As an illustrative example, the figure shows a scenario when C/N ratios are 20, 15 and 10 for POM, MAOM and microbes, respectively.

FIGURE 2 Comparison of C/N ratios of particulate organic matter (POM), mineral-associated organic matter (MAOM), and microbial biomass (microbe) across all ecosystems (N = 288) (a), and in forests (N = 64) (b), grasslands (N = 69) (c), and croplands (N = 146) (d). The plots display the individual C/N ratios, as well as their density distribution, means and standard deviations. *** indicates $p < 0.001$, * indicates $p < 0.05$.

FIGURE 3 Regressions between the C/N ratios of mineral-associated organic matter (C/N_{MAOM}) and microorganisms ($C/N_{Microbe}$), or particulate organic matter (C/N_{POM}) across all ecosystems (a) and separately for forests (b), grasslands (c), and croplands (d). The X-axis refers to the C/N ratio of microbial biomass or POM. The black dashed line shows the 1:1 relationship. A few data points representing C/N ratios of POM fall outside the range of the X-axis (> 40), as do a few MAOM data points for the Y-axis (> 25), and consequently are not shown here (see Table S2).

FIGURE 4 Microbial contribution to MAOM depends on ecosystem type (a) and soil depth (b). Abnormal points (with contribution equal to 0 or 100%) were excluded in this analysis. The box represents the upper and lower quartiles. The top and bottom whisker-line represent the maximum and minimum values, the black dot and nearby black number in the box represents the mean value, and the horizontal line in the box represents the median. The scattered points within the box and whisker-line display the individual contributions. The numbers in the parentheses indicate the sample size.

FIGURE 5 Relative importance of climate and soil properties to predict microbial contribution to MAOM by random forest analysis. The abnormal points (with contribution equal to 0 or 100%) were excluded in this analysis. Red bars indicate that the factor is positively correlated with the microbial contribution to MAOM; blue bars indicate that the factor is negatively correlated with the microbial contribution to MAOM. %IncMSE, percentage of increase of mean square error (%). MAP: mean annual precipitation, MAT: mean annual temperature.

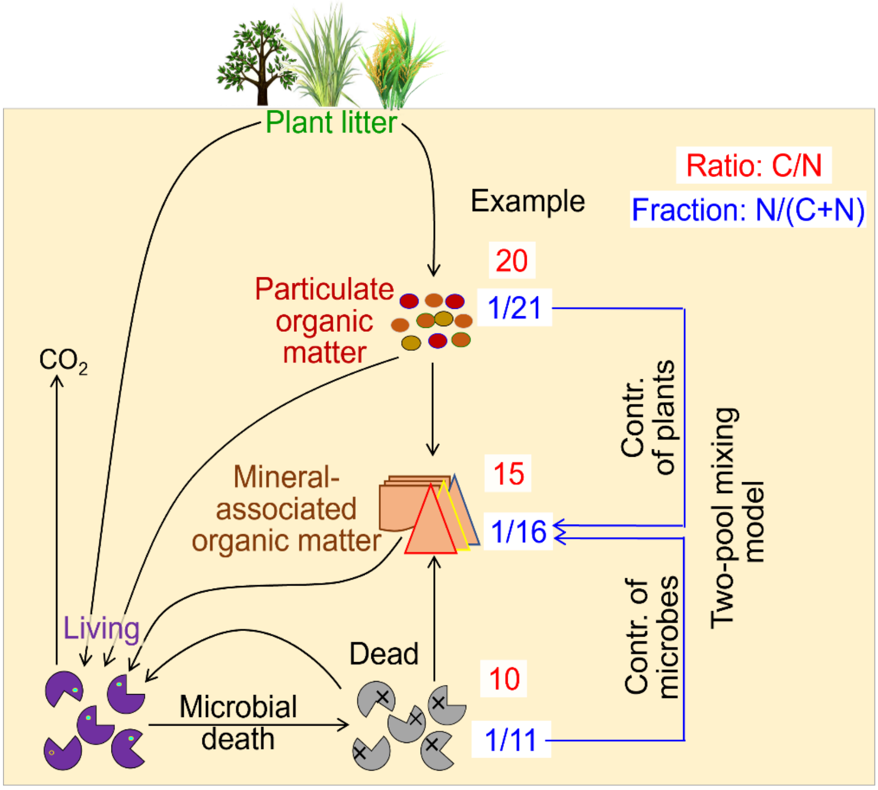

FIGURE 1

Quantify the sources of MAOM by stoichiometry

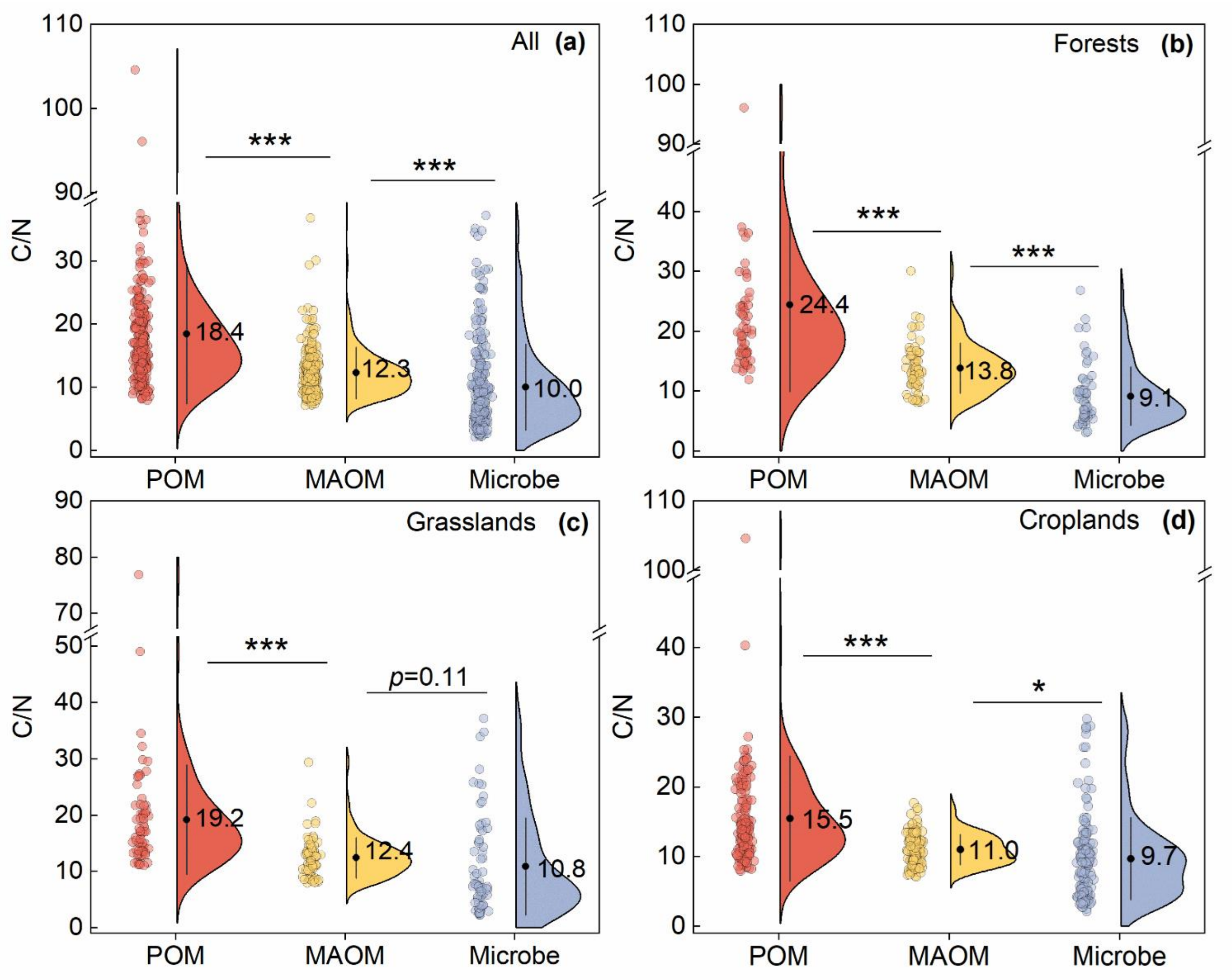

FIGURE 2

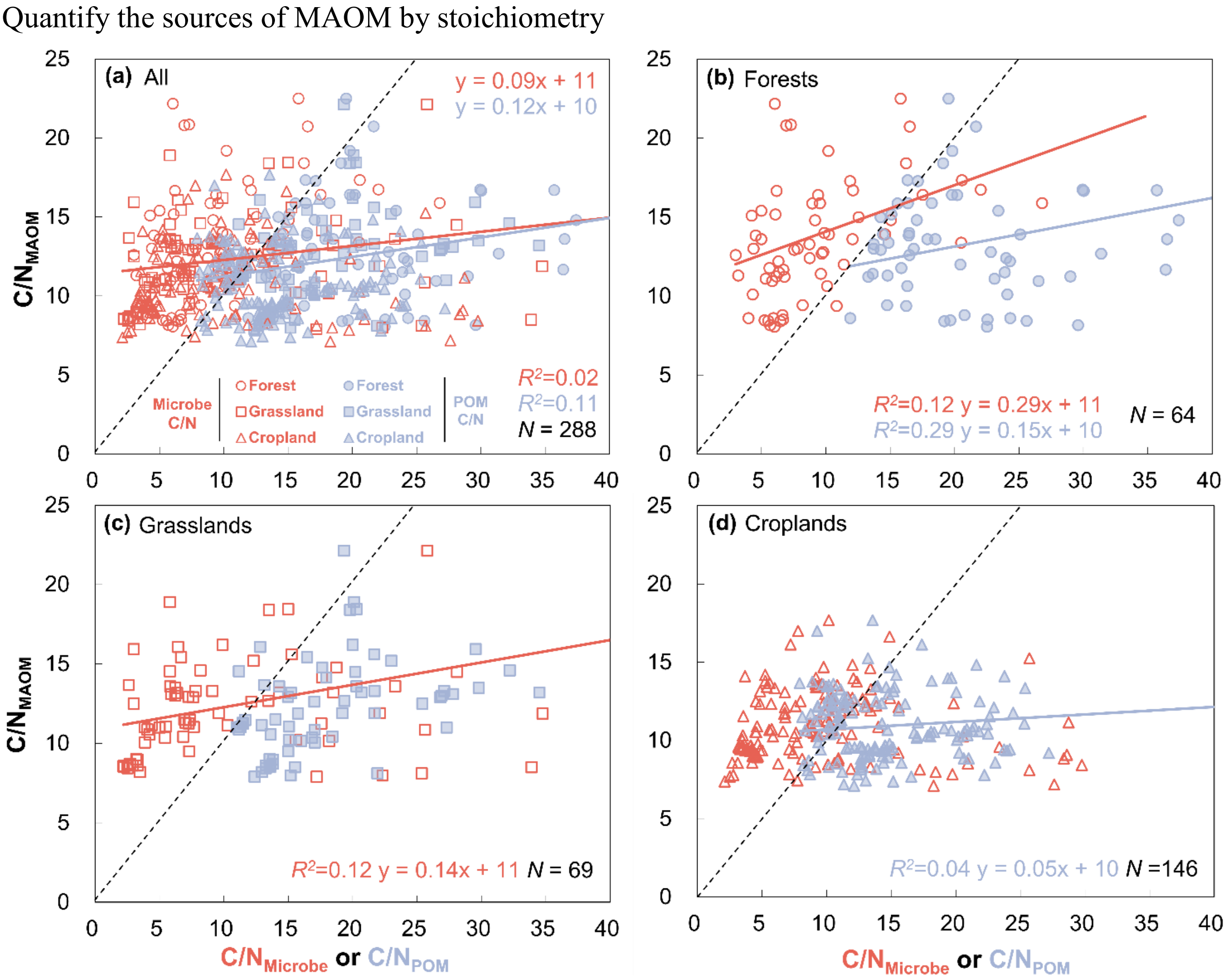
Quantify the sources of MAOM by stoichiometry
(a) All
y = 0.09x + 11
y = 0.12x + 10
Microbe C/N
Forest
Grassland
Cropland
Forest
Grassland
Cropland
POM C/N
R^2=0.02
R^2=0.11
N = 288
(b) Forests
R^2=0.12 y = 0.29x + 11
R^2=0.29 y = 0.15x + 10
N = 64
(c) Grasslands
R^2=0.12 y = 0.14x + 11 N = 69
(d) Croplands
R^2=0.04 y = 0.05x + 10 N =146
C/N_{MAOM}
$C/N_{Microbe}$ or C/N_{POM}
0
5
10
15
20
25
30
35
40

FIGURE 3

Quantify the sources of MAOM by stoichiometry

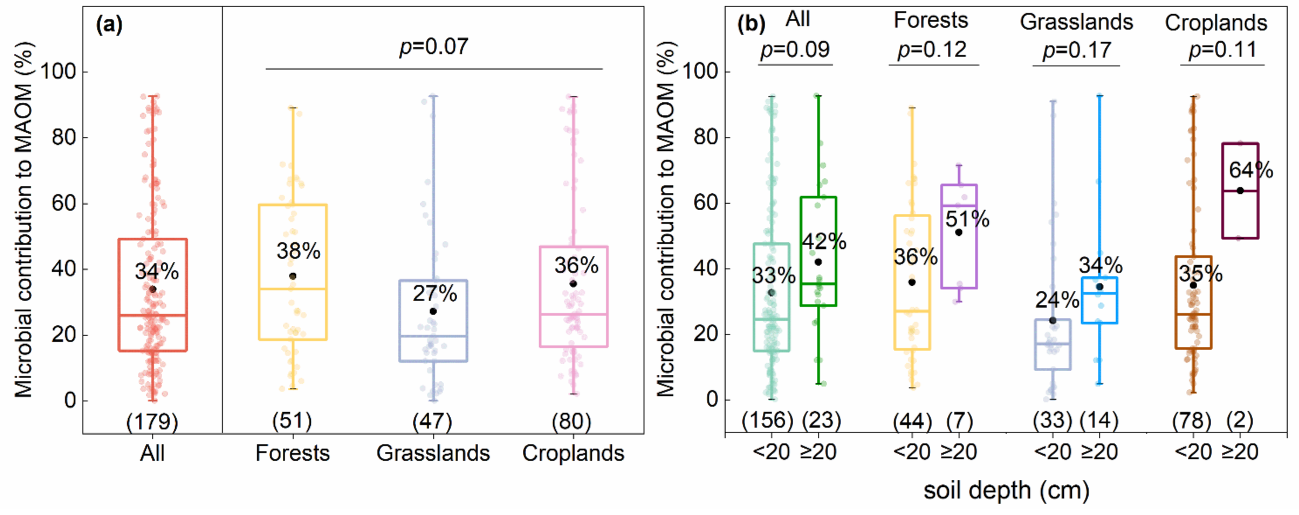

FIGURE 4

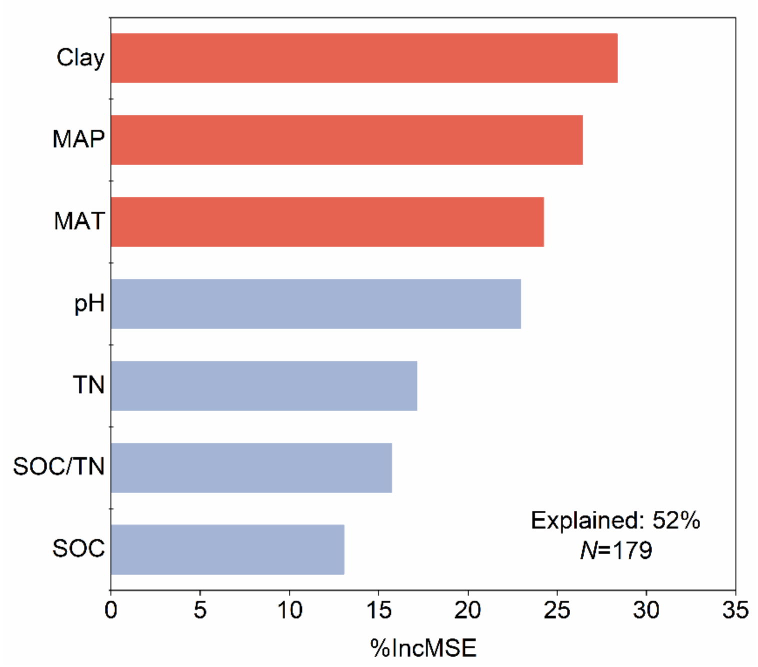

FIGURE 5