

## RESEARCH ARTICLE

# Nitrogen increases soil organic carbon accrual and alters its functionality

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## Abstract

Nitrogen (N) availability has been considered as a critical factor for the cycling and storage of soil organic carbon (SOC), but effects of N enrichment on the SOC pool appear highly variable. Given the complex nature of the SOC pool, recent frameworks suggest that separating this pool into different functional components, for example, particulate organic carbon (POC) and mineral-associated organic carbon (MAOC), is of great importance for understanding and predicting SOC dynamics. Importantly, little is known about how these N-induced changes in SOC components (e.g., changes in the ratios among these fractions) would affect the functionality of the SOC pool, given the differences in nutrient density, resistance to disturbance, and turnover time between POC and MAOC pool. Here, we conducted a global meta-analysis of 803 paired observations from 98 published studies to assess the effect of N addition on these SOC components, and the ratios among these fractions. We found that N addition, on average, significantly increased POC and MAOC pools by 16.4% and 3.7%, respectively. In contrast, both the ratios of MAOC to SOC and MAOC to POC were remarkably decreased by N enrichment (4.1% and 10.1%, respectively). Increases in the POC pool were positively correlated with changes in aboveground plant biomass and with hydrolytic enzymes. However, the positive responses of MAOC to N enrichment were correlated with increases in microbial biomass. Our results suggest that although reactive N deposition could facilitate soil C sequestration to some extent, it might decrease the nutrient density, turnover time, and resistance to disturbance of the SOC pool. Our study provides mechanistic insights into the effects of N enrichment on the SOC pool and its functionality at global scale, which is pivotal for understanding soil C dynamics especially in future scenarios with more frequent and severe perturbations.

## KEYWORDS

global change, meta-analysis, mineral-associated organic carbon, nitrogen deposition, particulate organic carbon, soil organic carbon

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## 1 | INTRODUCTION

The soil organic carbon (SOC) is the largest terrestrial C pool, which plays an important role in global C cycling and C-climate feedbacks (Bradford et al., 2016; Falkowski et al., 2000). Therefore, exploring the mechanisms of SOC formation, stabilization, and preservation has received considerable attention, especially in the context of global environmental change (Averill & Waring, 2018; Cotrufo et al., 2015; Sokol et al., 2019; Witzgall et al., 2021). A growing body of evidence over the past decades has indicated that we cannot understand these properties of SOC by considering it as a single, uniform entity due to its complex nature (Lavallee et al., 2020; Lugato et al., 2021; Trumbore, 2009). Fortunately, recent frameworks suggest that separating SOC into components characterized by different functioning, for example, particulate organic carbon (POC) and mineral-associated organic carbon (MAOC), is of great importance for understanding and predicting SOC dynamics and its response to global environmental change (Averill & Waring, 2018; Cotrufo et al., 2019; Lavallee et al., 2020).

It is well known that two components (POC vs. MAOC) of the SOC pool are very different in terms of their formation, persistence, and functioning (Lavallee et al., 2020). POC consists predominantly of lightweight fragments (e.g., large polymers) that are less decomposed, persists in soil via inherent biochemical properties and physical protection in aggregates (Cotrufo et al., 2019; Lavallee et al., 2020; Yu et al., 2022). MAOC, by contrast, is thought to consist mainly of single small molecules (e.g., low molecular weight compounds), generally protected from decomposition through chemical bonds between organic carbon and minerals and protection within micropores and microaggregates, thus resulting in MAOC being less accessible to decomposers and extracellular enzymes (Kögel-Knabner et al., 2008; Lavallee et al., 2020). Due to their different degree of protection against decomposition, MAOC, on average, persists longer than POC (Kögel-Knabner et al., 2008; Poelau et al., 2018). In addition, MAOC generally has a higher nutrient density than POC, because of the lower C:N ratio in MAOC relative to POC (Sokol et al., 2022; Williams et al., 2018). Therefore, it is assumed that changes in POC and MAOC pools can alter the functionality of the SOC pool (Sokol et al., 2022). Both POC and MAOC are likely to respond differently to global change, potentially leading to different effects on the SOC pool and its functionality, and hence the long-term storage of SOC, which is an area that needs urgent research (Kristensen et al., 2022).

Given that terrestrial C cycling is tightly coupled with nitrogen (N), it is reasonable to assume that global C cycling will be greatly impacted by the unprecedented reactive N deposition as a result of agricultural activities and fossil fuel combustion (Vitousek, 1994). Increased N deposition generally increases SOC storage, as elevated N can facilitate the production of plant biomass, and thus C inputs to soil (Chen, Luo, et al., 2018; Reay et al., 2008; Wan et al., 2021; Ye et al., 2018). However, this is not always the case. For example, elevated N has also been shown to decrease soil C storage (Mack et al., 2004; Neff et al., 2002). These conflicting results

among studies may, to a large extent, be ascribed to the different responses of various C fractions (e.g., POC vs. MAOC) to N deposition (Averill & Waring, 2018). When the positive effect of elevated N on plant growth overrides the stimulation of microbial decomposition, N enrichment can often accrue POC (Chen et al., 2020; Ye et al., 2018) and vice versa (Neff et al., 2002). However, N deposition can also cause soil acidification (Tian & Niu, 2015), which would affect MAOC dynamics through the following pathways. First, soil acidification can significantly suppress microbial metabolism. This may inhibit the formation of MAOC because microbial residues are thought to be important precursors for C incorporated in MAOC (Cotrufo et al., 2015; Liang et al., 2017), but see Craig et al. (2022). Additionally, accelerated leaching of base cations (e.g.,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{K}^{+}$ ) as a consequence of soil acidification plays an important role in regulating the formation of MAOC, by altering the sorption of C compounds to mineral surfaces (Averill & Waring, 2018; Ye et al., 2018). Finally, greater solubility of iron (Fe) and aluminum (Al) phases that co-occur with acidification would impact MAOC dynamics via co-precipitation reactions. Therefore, a full understanding of the fundamental mechanism regulating both POC and MAOC in the context of N deposition is pivotal for accurately predicting soil C dynamics and reducing model uncertainties in forecasting terrestrial C-climate feedbacks.

Although several studies of field investigation and model simulation have been conducted to investigate the effect of N enrichment on POC and MAOC (Averill & Waring, 2018; Chen et al., 2020; Keller et al., 2021; Ye et al., 2018), results differ among these studies and this hampers our ability to generalize. Therefore, further investigations across different ecosystems are critically required. Previous meta-analyses showed that N fertilization significantly increases both POC and MAOC pools (Rocci et al., 2021), or labile and non-labile C fractions (Chen, Li, et al., 2018), yet it remains unclear what is the underlying mechanism for these alterations. Additionally, we have a limited understanding of how these variations in POC and MAOC resulting from N enrichment regulate the functionality of the SOC pool. To fill these knowledge gaps, we compiled a dataset regarding the effect of experimental addition of N on POC and MAOC pools, the ratios among these fractions, and a wide range of experimental and environmental factors across different ecosystems. Importantly, we collect data on direct controls of POC and MAOC (e.g., plant and microbial biomass and enzyme activity), which were lacking in previous meta-analyses, and this allows for more direct assessment of the mechanistic underpinnings of POC and MAOC responses to N addition. By conducting a meta-analysis, we specifically focused on (1) the responses of POC and MAOC to experimental addition of N and the underlying mechanisms, and (2) how these variations would be translated to changes in the functionality of the SOC pool. In general, MAOC has a higher nutrient density, greater resistance to disturbance, and longer turnover time than POC (Grandy & Robertson, 2007; Kögel-Knabner et al., 2008; Sokol et al., 2022; Williams et al., 2018), even though in certain contexts, the turnover time of both C pools is approximately the same (Heckman et al., 2022). Given that the SOC pools might differ

between N addition treatment and control (no N addition), it seems reasonable to assess the changes in their functionality via the ratios among these SOC fractions (Heckman et al., 2022; Lavallee et al., 2020; Sokol et al., 2022). By using additional data sources, our meta-analysis builds on two to three times the number of studies compared to any previous such analyses.

## 2 | MATERIALS AND METHODS

### 2.1 | Data collection

On the basis of the Web of Science (WoS, <http://apps.webofknowledge.com/>) and China Knowledge Resource Integrated Databases (CNKI, [www.cnki.net/](http://www.cnki.net/)), we searched peer-reviewed articles that investigated the effects of N addition on POC and/or MAOC on April 20, 2021, without restriction on publication year. In the WoS, databases used for our search included WoS Core Collection, Current Contents Connect, Chinese Science Citation Database, Derwent Innovations Index, KCI-Korean Journal Database, MEDLINE and SciELO Citation Index. The keyword combinations used for topic searching were: ("nitrogen addition" OR "nitrogen deposition" OR "nitrogen enrichment" OR "fertiliz\*") AND ("mineral-associated organic matter" OR "mineral-associated OM" OR "MAOM" OR "mineral-associated organic carbon" OR "mineral-associated OC" OR "MAOC" OR "particulate organic matter" OR "particulate OM" OR "POM" OR "particulate organic carbon" OR "particulate OC" OR "POC" OR "mineral protection" OR "soil carbon fractions") AND ("terrestrial" OR "land" OR "soil"). We updated the search in December, 2021. In addition, we checked the references list of relevant reviews and meta-analyses to obtain the target articles (Chen, Li, et al., 2018; Lavallee et al., 2020; Rocci et al., 2021). Finally, our dataset was further supplemented by the dataset from a relevant meta-analysis (Rocci et al., 2021), which used the same definitions of POC and MAOC as used in this study. Our approach resulted in the inclusion of two to three times the number of studies as in previous such analyses (Table S1).

To avoid bias, the studies compiled in our dataset had to meet the following criteria: (1) Results came from a field experiment; (2) duration of the experiment was at least 1 year/growing season; (3) control and N addition treatments had to be established at the same site with similar microclimate, vegetation, and soil properties; (4) means and sample size had to be reported or could be derived from the article; (5) details of experimental N addition had to be provided, for example, magnitude, type, and duration. Given that diverse methods were adopted to separate POC and MAOC in these articles, it is difficult to make cross-study comparisons. To be included in our analysis, therefore, the soil samples had to be dispersed first using either sodium hexametaphosphate, sonication, or shaking with glass beads to ensure adequate dispersion of aggregates. Additionally, POC in these articles was defined as lighter than 1.6–1.85 g/cm<sup>3</sup> (separated by density) or larger than 50–63 μm (separated by size), and MAOC defined as heavier than 1.6–1.85 g/cm<sup>3</sup> or smaller than 50–63 μm

(Lavallee et al., 2020). The procedure of article selection followed the PRISMA guidelines (Figure S1). Finally, a total of 98 studies met these criteria (Figure S2) and were used for further analysis. For each study, we recorded POC and/or MAOC, and SOC if reported. If a study reported data for multiple soil layers, we only collected data for the uppermost layer. When some critical information was not reported in the original paper, we attempted to contact the corresponding author to obtain this information. If results were presented graphically, the software WebPlotDigitizer 4.1 (<https://automeris.io/WebPlotDigitizer/>) was used to digitize the data.

### 2.2 | Experimental and environmental variables

In order to explore the key moderators of N enrichment on the SOC pool, we extracted the information on N addition magnitude (g N m<sup>-2</sup> year<sup>-1</sup>), N type (inorganic N, organic N, and the mixture of both N), and experimental duration (year). The N addition magnitude and experimental duration was divided into different subgroups to make our analysis comparable to previous meta-analysis studies. Specifically, N addition magnitude was grouped by >15 (high level), 5–15 (medium level), and <5 g N m<sup>-2</sup> year<sup>-1</sup> (low level); experimental duration was grouped by >12 (long term), 4–12 (medium term), and <4 years (short term). Given the effects of mycorrhizal association on the C distribution between POC and MAOC pools (Cotrufo et al., 2019), we also tabulated mycorrhizal association (e.g., arbuscular mycorrhizae, AM; or ectomycorrhizae, ECM) of the dominant species at each experimental site according to the information provided in original publications, or using the latest FungalRoot database (Soudzilovskaia et al., 2019). Additionally, a wide range of environmental variables was also recorded in our dataset, including site locations (longitude and latitude), climate (mean annual precipitation, MAP and mean annual temperature, MAT), and ecosystem types (forest, grassland, and cropland). If the local climate data were not reported in the original paper, we obtained MAT and MAP from the WorldClim dataset (<http://www.worldclim.org/>) on the basis of longitude and latitude.

### 2.3 | Other ancillary variables

Given that plant, microbial, and geochemical factors play an important role in controlling SOC storage in response to experimental addition of N (Averill & Waring, 2018; Chen, Luo, et al., 2018; Chen et al., 2020; Cusack et al., 2011; Liang et al., 2017; Rowley et al., 2018; Ye et al., 2018), the following variables involved in SOC cycling were incorporated in our dataset (Table S2). (1) For soil-related variables, we extracted the dissolved organic carbon (DOC), ratio of soil carbon to nitrogen (C:N), soil total N (TN), dissolved inorganic N (DIN), soil base cations (e.g., Ca<sup>2+</sup>, Mg<sup>2+</sup>, Na<sup>+</sup>, and K<sup>+</sup>), and soil acid cation (Al<sup>3+</sup>). Because few studies have reported the responses of soil texture (e.g., clay and silt) and bulk density to N enrichment, we recorded these variables for each

study site. Data on soil texture and bulk density were obtained from the SoilGrids database (<https://www.isric.org/explore/soilgrids>) if they were not reported in the literature. (2) For plant communities, we recorded aboveground biomass (AGB) and belowground biomass (BGB). (3) For microbial communities, we recorded fungal biomass (FB), bacterial biomass (BB), microbial biomass (MB), microbial biomass C (MBC), and microbial biomass N (MBN). (4) For enzymatic activities, we recorded  $\beta$ -glucosidase (BG),  $\beta$ -1,4-N-acetyl-glucosaminidase (NAG), L-aminopeptidase (LAP), acid phosphatase (AP), peroxidase (PER), and phenol oxidase (POX). If a study reported POC and/or MAOC but without the ancillary variables mentioned above, we attempted to find matching ancillary variables from the papers published by the same research group. For example, the data about POC and MAOC were reported in Lu, Vitousek, et al. (2021) and Ye et al. (2018), while the matching ancillary data (e.g., FB, BB, MB, soil base cations, and  $Al^{3+}$ ) were published in Lu et al. (2014) and Chen et al. (2016), respectively. Additionally, we tried to obtain the ancillary data by directly contacting the corresponding author (e.g., Keller et al., 2021).

## 2.4 | Effect size and its variance

The natural log response ratio (lnRR) was used to evaluate the effect size of N addition on variables, a metric commonly used in meta-analysis (Hedges et al., 1999):

$$\ln RR = \ln(X_T/X_C) = \ln X_T - \ln X_C,$$

where  $X_C$  and  $X_T$  are the arithmetic mean values of the variables in the control and N addition treatments, respectively. The variance ( $v$ ) for each lnRR was calculated as follows:

$$v = \frac{SD_T^2}{N_T X_T^2} + \frac{SD_C^2}{N_C X_C^2},$$

where  $SD_C$  and  $SD_T$  are the standard deviation (SD) in the control and N addition treatments, respectively;  $N_C$  and  $N_T$  are the sample size in the control and N addition treatments, respectively. If a study reported standard error (SE), then the corresponding SD was calculated using the following equation:

$$SD = SE \times \sqrt{n},$$

where  $n$  is the sample size. If in a paper the POC was further separated into macro-POC (or coarse-POC, e.g., >250  $\mu$ m) and micro-POC (or fine-POC, e.g., 53–250  $\mu$ m), then their sum values were considered as the POC. The corresponding SD values for the control and treatment were calculated according to the method of error propagation (Lorber, 1986):

$$SD_{\text{sum}} = \sqrt{SD_a^2 + SD_b^2},$$

where  $SD_{\text{sum}}$  is the new SD for the sum values, with  $SD_a$  and  $SD_b$  as the values of each fraction of POC. In order to investigate the N effects on the functionality of the SOC pool, we calculated the ratios of MAOC to POC and MAOC to SOC when a study reported MAOC and SOC simultaneously. Similarly, the SD values for these ratios were calculated following the method of error propagation (Lorber, 1986):

$$SD_{\text{ratio}} = x \sqrt{\left(\frac{SD_{\text{maoc}}}{\text{Mean}_{\text{maoc}}}\right)^2 + \left(\frac{SD_{\text{soc}}}{\text{Mean}_{\text{soc}}}\right)^2},$$

where  $SD_{\text{ratio}}$  is the SD value for the newly calculated ratio;  $x$  the calculated mean value of the ratio of MAOC to SOC;  $\text{Mean}_{\text{maoc}}$  and  $\text{Mean}_{\text{soc}}$  are the mean values of MAOC and SOC, with  $SD_{\text{maoc}}$  and  $SD_{\text{soc}}$  as the SD values of MAOC and SOC, respectively. Since not all studies reported SD, variances or SE in our dataset (7.43% observations with missing SE or SD), we used the "Bracken1992" approach to impute missing SD based on the coefficient of variation from all complete cases, which were performed using the R package *metagear* (Lajeunesse, 2016). Density distributions of the response ratios (lnRR) of SOC fractions and the ratios among these fractions are presented in Figure S3.

## 2.5 | Statistics

First, we used a mixed-effect model with restricted maximum likelihood approach (REML) to calculate the weighted mean of lnRR ( $\ln \overline{RR}$ ), which was conducted in the R package *metafor* (Viechtbauer, 2010). Specifically, the  $\ln \overline{RR}$  was calculated as:

$$\ln \overline{RR} = \frac{\sum_{i=1}^m w_i \ln RR_i}{\sum_{i=1}^m w_i}$$

where  $m$  represents the number of comparisons in the group,  $\ln RR_i$  is the effect size of the  $i$ th study. The weighing of the  $i$ th study ( $w_i$ ) was calculated as:

$$w_i = \frac{1}{v_i},$$

$$v_i = v + \tau^2,$$

where  $v_i$  represents the variance of study ( $i$ ),  $v$  indicates the variance within study, and  $\tau^2$  is the variance between studies. Given that some studies contributed more than one effect size, we ran a hierarchical meta-analysis to control for non-independence in the data. To deal with this, the variable "study" was treated as a random factor in the mixed-effect model. Although we primarily focused on the uppermost layer of soil, the sampling depth of this soil layer varied among studies in our dataset. Therefore, we also included "sampling depth" as a random factor in this meta-analysis. The effects of N addition were considered significant if the 95% confidence intervals

did not overlap with zero. To ease interpretation, the  $\ln\text{RR}$  was back-transformed and expressed as percentage change (%):

$$\text{Percentage change (\%)} = (e^{\ln\text{RR}} - 1) \times 100$$

Second, we attempted to identify the most important predictors for the effects of N addition on variables in a mixed-effects meta-regression model using the *glmulti* package in R. The importance of a particular predictor was equal to the sum of the Akaike weights for the models that included the predictor. If a predictor was included in models with large Akaike weights, then it will receive a high importance value (Terrer et al., 2016). A cutoff of 0.8 was set to differentiate between essential and non-essential predictors (Terrer et al., 2016). We also assessed the relationships between SOC fractions (e.g., POC and MAOC) and variables of soil, plant community, microbial community, and enzymatic activity (Table S2) using regression analysis.

Third, the quality of this meta-analysis was checked according to the checklist of quality criteria proposed by Koricheva and Gurevitch (2014). To examine the possibility that publication bias affected the results of this meta-analysis, the Rosenthal fail safe number (fail-safe number) was calculated using the *fsn* function (Table S4), and funnel plots were created using the *funnel* function (Filazzola et al., 2020) (Figure S10). The trim and fill method was adopted to correct publication bias using the *trimfill* function if necessary (Duval & Tweedie, 2000). In addition, we also tested the robustness of our results by conducting *leave-one-out* analysis using the *leave1out* function (Figure S11). All analyses were conducted in R (R Development Core Team, 2019).

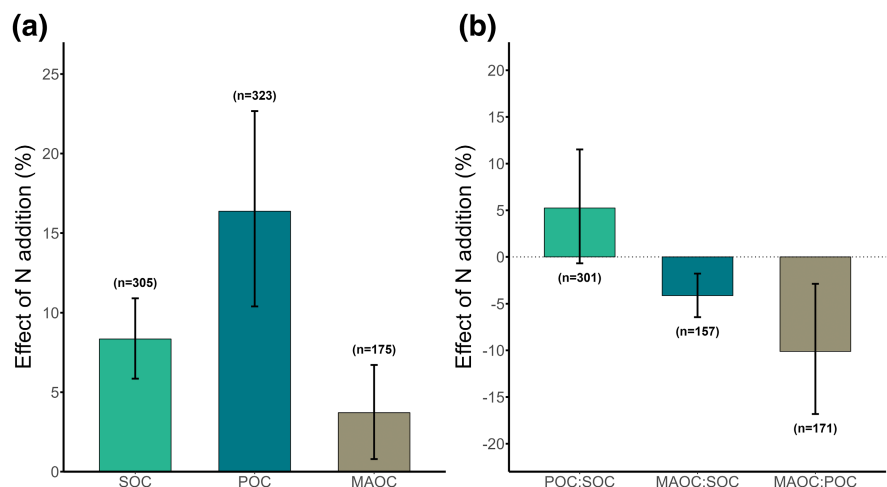
### 3 | RESULTS

Across all studies, N enrichment significantly increased POC, MAOC, and SOC concentrations by 16.4% (95% CI, 10.4%–22.7%,  $p < .001$ ), 3.7% (95% CI, 0.8%–6.7%,  $p < .05$ ), and 8.3% (95% CI, 5.9%–10.9%,  $p < .001$ ), respectively (Figure 1a), but the magnitude of this positive effect on POC was much higher than that of MAOC (Figure 1a). We found that these effects were not regulated by ecosystem type,

N type, mycorrhizal type, N magnitude, or experimental duration (Figure 2 and Figure S4). Consistent with this analysis, none of the predictors reached the threshold of 0.8 for the effects of N enrichment on POC and MAOC (Figure 3). Results of regression analysis showed that the response ratios of POC to N enrichment were positively related to aboveground biomass, dissolved organic C, and measures of N availability (e.g.,  $\beta$ -1,4-*N*-acetyl-glucosaminidase, L-aminopeptidase, soil total N, dissolved inorganic N, and microbial biomass N) (Figure 4a), but negatively correlated with bacterial biomass and microbial biomass C (Figure 4a). However, we found that changes in MAOC with N enrichment were positively correlated with changes in microbial biomass and soil total N (Figure 4b), but negatively related to soil base cations, for example,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ , and  $\text{K}^{+}$  (Figure 4b). Given that aboveground residues management in cropland might affect SOC dynamics, we examined whether removal of aboveground residues affects the responses of POC and MAOC to N addition. Results showed that there were no significant differences between removed and retained residues in cropland (Figure S5).

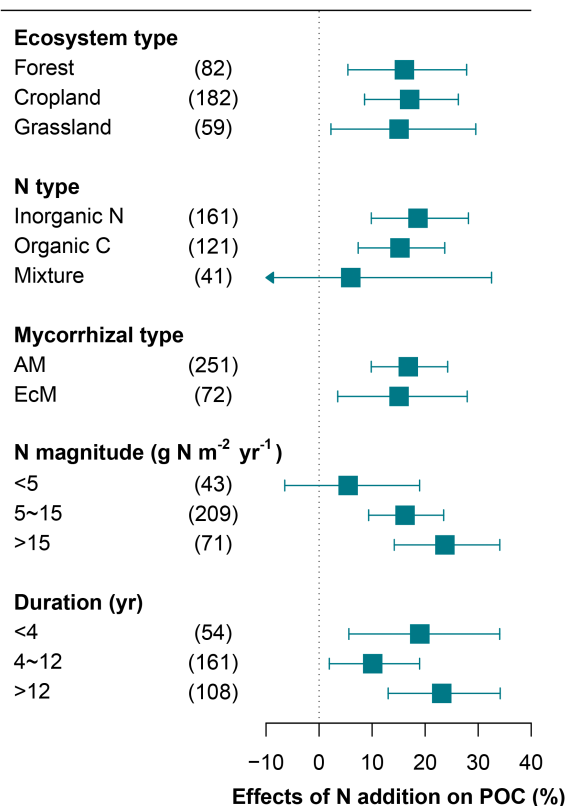
On average, N enrichment significantly decreased the ratio of MAOC to SOC and the ratio of MAOC to POC by 4.1% (95% CI, –6.4% to –1.8%,  $p < .05$ ) and 10.1% (95% CI, –16.8% to –2.9%,  $p < .01$ ), respectively (Figure 1b). However, no changes were observed for the effects of N enrichment on the ratio of POC to SOC (mean, 5.24%; 95% CI, –0.79% to 11.5%;  $p = .19$ ; Figure 1b). These effects of N enrichment were consistent regardless of ecosystem type, N type, mycorrhizal type, N magnitude, and experimental duration (Figure S6), which was further confirmed by the analysis of model selection (Figure S7). Response ratios of MAOC:POC were positively correlated with those of microbial biomass and microbial biomass C, but negatively correlated with aboveground biomass, L-aminopeptidase, and dissolved inorganic N (Figure S8a). However, we found that the response ratio of MAOC:SOC was negatively correlated with changes in  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ , and L-aminopeptidase (Figure S8b).

Meta-analysis of the effect of N addition on the variables involved in SOC cycling showed that aboveground biomass significantly increased, but no effect was observed for belowground biomass (Figure S9). For soil variables, experimental addition of N significantly elevated soil total N, dissolved inorganic N, and  $\text{Al}^{3+}$ , but decreased soil



**FIGURE 1** Overall effects of N addition on SOC, POC, and MAOC pools (a), and the ratios of POC to SOC, MAOC to SOC, and MAOC to POC (b). Means and 95% confidence intervals (CIs) are given. The numbers above (a) or below (b) the CIs indicate the sample size. MAOC, mineral-associated organic carbon; POC, particulate organic carbon; SOC, soil organic carbon.

## (a) POC



## (b) MAOC

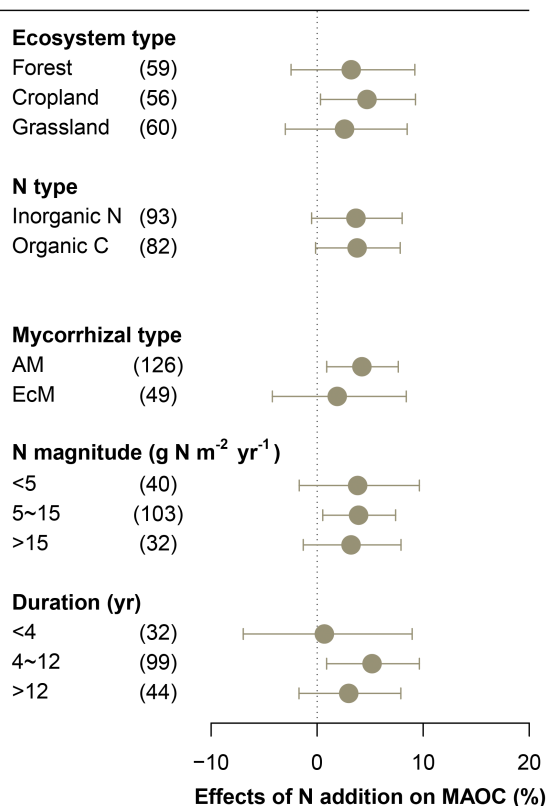


FIGURE 2 Meta-analysis of the effect of N addition on POC (a) and MAOC (b) across different factors. Squares and circles represent mean responses of POC and MAOC, respectively. Full bars represent the 95% confidence interval.

pH, extractable Ca<sup>2+</sup>, and extractable Mg<sup>2+</sup> (Figure S9). In addition, we found that N addition significantly increased microbial biomass, and the activities of  $\beta$ -glucosidase and L-aminopeptidase (Figure S9).

Results of fail-safe number showed that the fail-safe number for each variable (except for the ratio of MAOC to SOC) was much higher than their corresponding 5 K + 10 (Table S4), which indicated that no publication bias was observed for these variables. Results of funnel plots showed that there was no evidence of publication bias for these variables, except for POC and the ratio of POC to SOC (Figure S10). Therefore, effects of N enrichment on POC and POC:SOC were adjusted using a trim-and-fill method. According to *leave-one-out* analysis, no significant differences for these results were observed, suggesting that our findings are unlikely to be driven by a single influential study (Figure S11).

## 4 | DISCUSSION

### 4.1 | Positive effect of N enrichment on the POC pool

Our results demonstrate that experimental addition of N significantly increased the POC pool. This positive effect has been previously documented across different ecosystems (Chen et al., 2020; Keller

et al., 2021; Ye et al., 2018) and in meta-analyses (Chen, Li, et al., 2018; Rocci et al., 2021). However, our coupled plant and microbial data allow us to better evaluate the mechanisms underlying this response. Given that POC predominantly consists of lightweight fragments that are relatively undecomposed, stimulation of plant productivity with N enrichment (e.g., aboveground residues and roots) has been suggested to increase POC pool (Ye et al., 2018). Similarly, we also found that N-induced changes in POC were positively related to aboveground biomass with experimental addition of N. However, no significant effect of N addition on root biomass was found in this study, which contrasts to recent meta-analyses that reported positive effects (Xu et al., 2021; Yang et al., 2022). This can be attributed to the fact that the sample size (31 samples) in our dataset is too small to detect these significant changes, because we only compiled studies which had to also report POC and/or MAOC. Therefore, we cannot exclude the role of root biomass in regulating the response of POC to N enrichment, and more studies are needed to test this potential mechanism. Besides, we found no significant effect of residues management (removed vs. retained) on the responses of POC and MAOC to N addition in cropland, which may indicate that aboveground biomass can not only affect SOC dynamics directly (e.g., residues inputs), but indirectly by allocating more photosynthetic products (e.g., in the form of root production and exudates) belowground.

Stimulating plant growth with N enrichment could increase labile matter inputs to soil as well (e.g., root exudates), which would

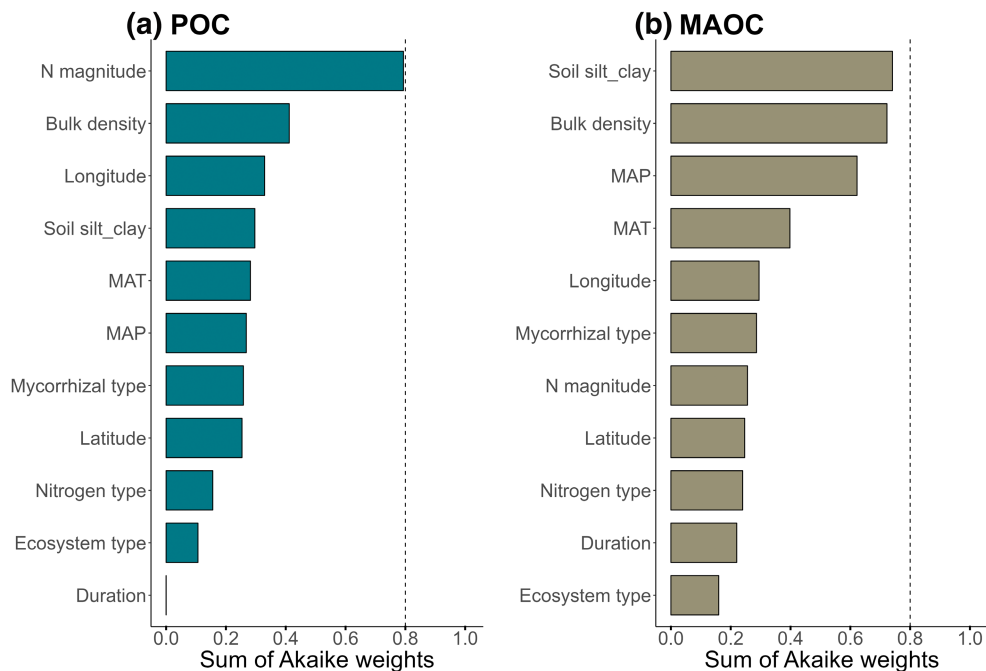


FIGURE 3 Model-averaged importance of the predictors for the effect of N addition on POC (a) and MAOC (b). Cutoff is set at 0.8 in order to differentiate among the most important predictors.

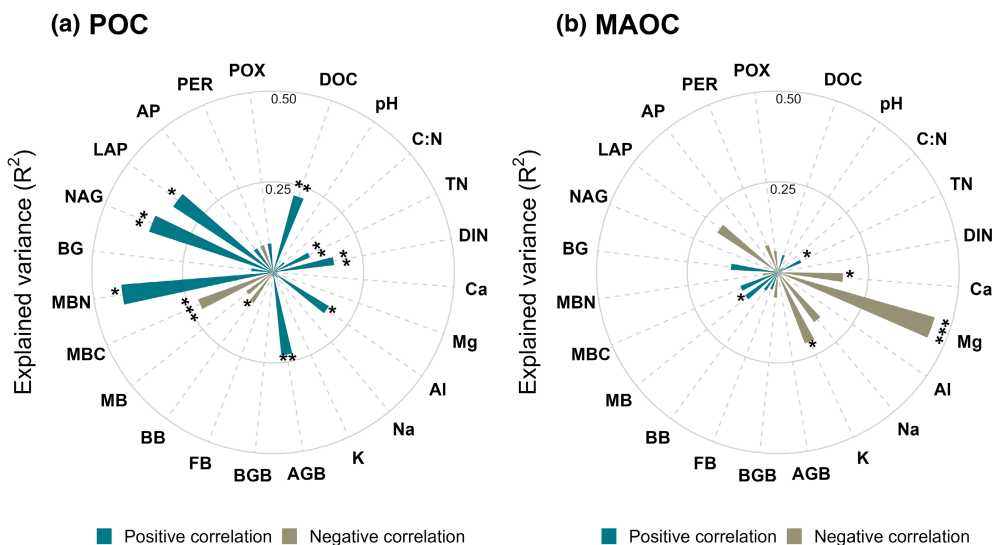


FIGURE 4 Relationships between the responses (lnRR) of SOC fractions (a, POC; b, MAOC) and soil (e.g., DOC, soil pH, soil C:N, TN, DIN, Ca, Mg, Al, Na, K), plant community (e.g., AGB and BGB), microbial community (e.g., FB, BB, MB, MBC, MBN), and enzymatic activity (e.g., BG, NAG, LAP, AP, PER, POX) related variables. DOC, dissolved organic C; TN, soil total N; DIN, dissolved inorganic N; Ca, extractable  $\text{Ca}^{2+}$ ; Mg, extractable  $\text{Mg}^{2+}$ ; Al, extractable  $\text{Al}^{3+}$ ; Na, extractable  $\text{Na}^+$ ; K, extractable  $\text{K}^+$ ; AGB, aboveground biomass; BGB, belowground biomass; FB, fungal biomass; BB, bacterial biomass; MB, microbial biomass; MBC, microbial biomass C; MBN, microbial biomass N; BG,  $\beta$ -glucosidase; NAG,  $\beta$ -1,4-N-acetyl-glucosaminidase; LAP, L-aminopeptidase; AP, acid phosphatase; PER, peroxidase; and POX, phenol oxidase. \* $p < .05$ ; \*\* $p < .01$ ; \*\*\* $p < .001$ .

alleviate N limitation of the microbial community and facilitate the production of extracellular enzymes; thus accelerating the decomposition of POC by priming effect (Cusack et al., 2011; Liu et al., 2020; Neff et al., 2002; Schimel & Bennett, 2004). Interestingly, we found that N-induced increases in POC were also positively correlated with changes in activities of  $\beta$ -1,4-N-acetyl-glucosaminidase and L-aminopeptidase, both of which play an important role in microbial

ex vivo modification of complex plant polymers into simpler plant compounds (Liang et al., 2017). These extracellular enzymes can effectively break down macromolecules from plants into smaller fragments (Liang et al., 2017), part of which can be directly used by microbes (Sinsabaugh et al., 2009), promoting the formation of microbial necromass (see below). The remaining material (partially decomposed) that has undergone ex vivo modification would be

accumulating in soils (Angst et al., 2017), and contributing to the POC formation (Ye et al., 2018). Because the quantity of existing POC is determined by the balance between inputs of plant residues and C loss induced by microbial decomposition (Averill & Waring, 2018), we suggest that N-induced C inputs from plant residues override the C loss by microbial decomposition in this dataset.

## 4.2 | Positive effect of N enrichment on the MAOC pool

Consistent with previous studies (Chen, Li, et al., 2018; Cusack et al., 2011; Lu, Vitousek, et al., 2021; Neff et al., 2002; Rocci et al., 2021), we found that experimental addition of N significantly increased the MAOC pool. However, negative effects of N enrichment on MAOC have also been reported in recent studies (Chen et al., 2020; Ye et al., 2018). When it comes to the responses of MAOC to N enrichment, it is necessary to understand the factors controlling its formation (Averill & Waring, 2018; Lavalley et al., 2020). Although similar to POC, plant and microbial communities are generally identified as key factors influencing MAOC dynamics (Averill & Waring, 2018; Cotrufo et al., 2013; Liang et al., 2017), the net effect of N addition on MAOC cannot be predicted from these effects alone (Chen et al., 2020; Ye et al., 2018). Rather, MAOC accrual is also strongly controlled by soil minerals (Kleber et al., 2021; Rowley et al., 2018; Ye et al., 2018). N-induced changes in leaching of base cations (e.g.,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ , and  $\text{K}^{+}$ ) and greater solubility of Fe and Al phases can also regulate the sorption and co-precipitation reactions that affect MAOC formation and preservation (Chen et al., 2020; Keller et al., 2021; Rasmussen et al., 2018; Rowley et al., 2018; Ye et al., 2018). Therefore, we evaluated the role of plants, microbes, and soil minerals in MAOC responses to N enrichment.

First, it is well known that one of the major pathways of MAOC formation is the mineral adsorption of relatively low molecular weight compounds (Lehmann & Kleber, 2015). These low molecular weight compounds not only can be produced by exoenzyme depolymerization of plant litter (ex vivo pathway) but also leach from plant litter directly (Liang et al., 2017; Sanderman et al., 2014). Consequently, increased plant productivity with N enrichment could promote the production of low molecular weight compounds, which would contribute to the MAOC formation. In addition, given that N-rich proteinaceous compounds play a crucial role in the formation of organo-mineral associations (Kleber et al., 2007; Possinger et al., 2020), promotion of N-rich compounds in plant litter with N enrichment (Li et al., 2019) would thus stimulate the formation of MAOC. Soil texture (especially clay and silt contents) has been traditionally deemed as the main control of SOC sequestration, given its control on MAOC formation (Hassink, 1997). However, we found no significant effect of soil initial clay and silt on the response of MAOC to N enrichment. Although soil C sequestration in some cases could be explained by the amount of soil clay and silt (Chen et al., 2019; Cotrufo et al., 2019), in other cases, different factors may be at work at the same time (Averill & Waring, 2018; Cotrufo & Lavalley, 2022;

Craig et al., 2021). Recent studies proposed that only 21%–42% of mineral surface associated with organic material, leaving most of the mineral surface uncovered (Cotrufo & Lavalley, 2022; Georgiou et al., 2022; Kopittke et al., 2020; Vogel et al., 2014), potentially indicating reduced importance of soil texture for MAOC formation.

Second, because microbial products (e.g., microbial exudates and necromass) are important precursors for the formation of MAOC (Cotrufo et al., 2015; Kallenbach et al., 2016; Liang et al., 2017), experimental addition of N could affect MAOC by influencing microbial communities. In general, when microbes are N limited, N enrichment would stimulate microbial biomass, thus facilitating the accumulation of necromass (Fan et al., 2020); however, if microbes are not limited by N availability, then N addition should have no effect (Averill & Waring, 2018). Furthermore, N availability has the potential to increase the osmotic potential and availability of toxic metals (e.g.,  $\text{Al}^{3+}$ ), which would, to a large extent, restrain microbial respiration and decomposition of microbial products, finally accelerating the accumulation of microbial necromass (Treseder, 2008; Zhang et al., 2018). However, it should be noted that while N deposition elevates N availability, it can also cause soil acidification, which was confirmed at both the local and global scales (Chen et al., 2016; Lu et al., 2014; Tian & Niu, 2015; Wan et al., 2021; Ye et al., 2018). When N enrichment exceeds the demand of plant for N, the conversion of  $\text{NH}_4^+$  to  $\text{NO}_3^-$  occurs (nitrification), a well-known acidification process (Matson et al., 1999). If the declines of soil pH with N enrichment exceed the threshold of acidification tolerance of microbes, it will then slow down the growth and decomposition of microorganisms. Importantly, this effect is independent of any effect of N enrichment on nutrient-limiting status of the microbial communities (Averill & Waring, 2018). N-induced changes in microbial communities and soil pH can also suppress microbial synthesis of oxidative enzymes (Chen, Luo, et al., 2018; Fog, 1988; Jian et al., 2016; Sinsabaugh, 2010), and consequently contribute to the accumulation of MAOC (Cusack et al., 2011).

Finally, experimental addition of N has the potential to regulate the formation and preservation of MAOC via the sorption and co-precipitation reactions (e.g.,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Fe}^{3+}$  and  $\text{Al}^{3+}$ ) that co-occur with soil acidification (Ye et al., 2018). Previous studies suggested that greater solubility of Fe and Al phases resulting from the declines of soil pH increases the stabilization of C in organo-mineral associations that may protect C from microbial exploitation (Gu et al., 1994; Vogel et al., 2014; Ye et al., 2018). Unfortunately, there is not yet enough available data to explore these linkages between the formation of MAOC and exchangeable  $\text{Fe}^{3+}$  and  $\text{Al}^{3+}$  in the context of soil acidification, and this knowledge is critically needed to assess the potential mechanism in MAOC formation. Given that losses of polyvalent cations (e.g.,  $\text{Ca}^{2+}$ ) can facilitate the release of organic C adsorbed on minerals via a decrease in polyvalent cation bridging (Rowley et al., 2018; von Lutzow et al., 2006), increased leaching of base cations with soil acidification might be detrimental to the formation and preservation of MAOC (Chen et al., 2020; Ye et al., 2018). However, we found that soil acidification-induced depletion of polyvalent cations was negatively correlated with changes in MAOC. This unexpected phenomenon might result from the fact that the dominance

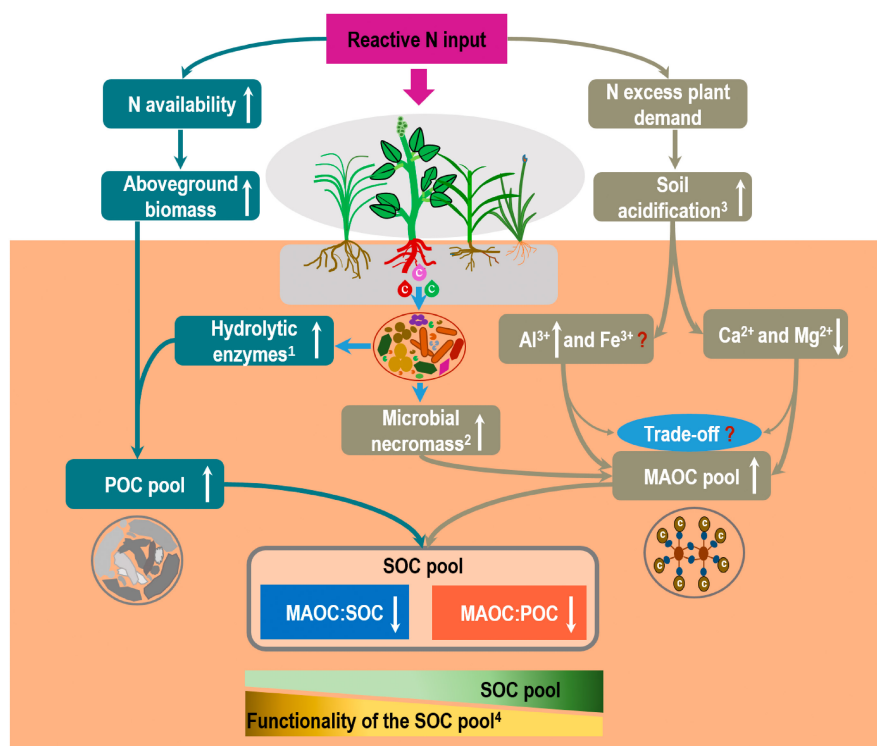


of the sorption mechanism during MAOC formation is context dependent. For example, MAOC formation in neutral and alkaline soils might be dominated by the interaction between organic carbon and polyvalent cation bridges, for example,  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  (Oades, 1988; von Lutzow et al., 2006), while co-precipitation reaction with Fe and Al phases might dominate in acidic soils (Ye et al., 2018). However, we lack sufficient data to explore these potential mechanisms. Therefore, with increasing soil acidification as a consequence of N enrichment, the stabilization mechanism of MAOC might shift from being dominated by polyvalent cation bridging (e.g., Ca-bound C) to organo-mineral associations, for example,  $\text{Fe}^{3+}$  and  $\text{Al}^{3+}$  (Rowley et al., 2018; Ye et al., 2018). Overall, we suggest that the trade-offs between depletion of cation bridging-C and increase of C-mineral complexes in the context of soil acidification should be ubiquitous, which is an important stabilization mechanism for maintaining the MAOC pool.

### 4.3 | Effect of N enrichment on the SOC pool and its functionality

In general, experimental addition of N significantly increased both POC and MAOC pools; but the magnitude for POC was much higher

than for MAOC (Figure 1a). These results could be attributed to different formation processes and controlling factors of these two components. In addition, the lower N costs for POC than MAOC formation might also contribute to the divergent responses of these components to N enrichment (Kicklighter et al., 2019; Lugato et al., 2021). From this vantage point, more C would be sequestered in soil in the form of POC under scenarios of accelerating N deposition (Chen et al., 2020; Ye et al., 2018). It is generally assumed that extreme climate events are more frequent and human-caused disturbances are intensifying, both of which may increase the vulnerability of soil carbon (Anderegg et al., 2020; IPCC, 2021). Therefore, it is highly necessary to shift the view from a highlight on immediate carbon storage to a focus on the persistence of soil C (Kristensen et al., 2022). Given that POC is expected to have a higher vulnerability to environmental changes than MAOC (Lugato et al., 2021), changes in the ratio of MAOC to POC (or MAOC to SOC) resulting from N deposition may potentially regulate the turnover time of the SOC pool (Sokol et al., 2022). In this study, we found that experimental addition of N significantly decreased the ratios of MAOC to SOC and MAOC to POC, which might be indicative of a lower nutrient density, resistance to disturbance, and turnover time (Figure 5). Similar results have also been reported in a previous study (Fang



**FIGURE 5** Graphical summary of the key findings with respect to the effects of N addition on the SOC pool (e.g., POC and MAOC pools) and the ratios among these fractions (MAOC:SOC and MAOC:POC). Although increased leaching of base cations (e.g.,  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ ) with soil acidification might be detrimental to the formation and preservation of MAOC, acidification-induced greater solubility of  $\text{Al}^{3+}$  and  $\text{Fe}^{3+}$  could increase the stabilization of C in organo-mineral associations. Therefore, the trade-offs between depletion of cation bridging-C and increase of C-mineral complexes under global N enrichment scenarios should be ubiquitous, a circumstance which has the potential to be an important mechanism for maintaining the MAOC pool under global N enrichment. Up- and downward arrows indicate increases and decreases in the respective variables in response to experimental addition of N, respectively. Question marks represent that we lack sufficient data to test this hypothesis. 1—Jian et al. (2016); 2—Hu et al. (2022); 3—Tian and Niu (2015); 4—functionality of the SOC pool, for example, nutrient density, turnover time, and resistance to disturbance.

et al., 2014). Taken together, our results suggest that although global N enrichment would increase soil C accrual, it might alter the functionality of the SOC pool to some extent (Figure 5).

#### 4.4 | Limitations and implications for future research

While our study provides important insights into the effects of N enrichment on the SOC pool and its functionality at global scale, some limitations still exist. First, most studies included in this meta-analysis were conducted in China, Europe, and America (Figure S1). Unequal geographical distribution of studies necessarily leads to certain limitations in our conclusions. Second, simulated N deposition was applied directly to soil in most forest experiments, which ignores the retention of N by the forest canopy (Lu, Kuang, et al., 2021). These results may overestimate the influence of N enrichment on SOC dynamics. Third, given that soil minerals play a critical role in MAOC formation (Kleber et al., 2021; Rowley et al., 2018; Ye et al., 2018), we attempted to explore how N-induced changes in base cations (e.g.,  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ ) and solubility of metal phases (e.g., Al and Fe) regulate MAOC formation. Unfortunately, the limited data available on these variables in the dataset hamper our ability to test the related mechanisms. To date, a great deal of attention has been focused primarily on the effects of plant and microbial communities on MAOC formation (Averill & Waring, 2018; Cotrufo et al., 2013; Liang et al., 2017), but the net effect of N addition on MAOC seemingly cannot be explained by these effects alone (Chen et al., 2020; Cusack et al., 2011; Ye et al., 2018). Thus, further investigation of MAOC formation in response to soil minerals in the context of N enrichment should be a priority of future studies. The framework of POC versus MAOC has been incorporated in some newer models (Fatichi et al., 2019; Robertson et al., 2019). Given that both POC and MAOC respond differently to N enrichment and these responses are associated with different regulators, we believe that it may be better to model POC and MAOC separately in the context of unprecedented reactive N deposition (Averill & Waring, 2018). By doing so, we can parameterize and validate models more easily, and thus predict the response of SOC to N enrichment more accurately.

## 5 | CONCLUSIONS

Our meta-analysis evaluated the responses of two functional components of SOC (POC vs. MAOC) and the ratios of MAOC to POC and MAOC to SOC to experimental addition of N. Both POC and MAOC pools are significantly increased by N enrichment, but the magnitude of this positive effect on POC is much higher than that on MAOC. However, experimental addition of N remarkably decreases the ratios of MAOC to POC and MAOC to SOC. Given that MAOC generally has higher nutrient density, greater resistance to disturbance, and longer turnover time than POC (Kögel-Knabner et al., 2008; Lavallee et al., 2020; Poeplau et al., 2018; Sokol et al., 2022; Williams

et al., 2018), our results suggest that although unprecedented reactive N deposition could facilitate soil C sequestration to some extent, it might alter the functionality of the SOC pool (Figure 5), especially in a future with increasing ecosystem perturbations (Rillig et al., 2019). This study is the first meta-analysis to directly relate these POC and MAOC responses to plant and microbial responses, which is critically important for us to understand soil C dynamics under ongoing global environmental change.

#### AUTHOR CONTRIBUTIONS

Bo Tang conceived the idea for the study. Bo Tang and Matthias C. Rillig designed this study. Bo Tang and Katherine S. Rocci collected data. Bo Tang and Anika Lehmann analyzed data. Bo Tang wrote the first draft, and all co-authors contributed to the revisions.

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#### CONFLICT OF INTEREST

The authors declare that they have no competing interests.

#### DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in figshare at <https://doi.org/10.6084/m9.figshare.21802096>.

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## SUPPORTING INFORMATION

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