Nitrogen addition-driven soil carbon stability depends on the fractions of particulate and mineral-associated organic carbon

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Abstract

Nitrogen (N) deposition greatly affects soil carbon (C) fractions, triggering changes in soil organic carbon (SOC) persistence and functionality. However, the responses of soil C fractions to N deposition remain unclear on a global scale. Here, we conducted a meta-analysis of 69 publications and explored the response of C fractions (particulate organic carbon, POC; mineral-associated organic carbon, MOC) to N addition. We found that N addition significantly increases the POC and MOC pool, yet the large rise in the fraction of POC (fPOC) and the decline in the fraction of MOC (fMOC) were observed, suggesting that N addition enhances soil C pool but decreases soil C stability globally. Moreover, the response ratios of POC and MOC were positively correlated with the duration of N addition. For soil C sequestration, POC was the most important predictor under short-term N addition, while the MOC jointly contribute to the C accumulation after long-term N addition. Overall, our study provides solid evidence that N addition would reduce the soil C stability mainly depending on the POC change and proposes a novel approach to predict the soil C-climate feedback for Earth System Models.

1. Introduction

Nitrogen (N) deposition has risen significantly over the last decades and is expected to increase in the future (Kuypers et al., 2018). Given the firm coupling between terrestrial C and N cycles, the N deposition has dramatically affected the C cycle and its feedback to climate change (Liu and Greaver, 2010; Greaver et al., 2016). Soil is the largest C reservoir in terrestrial ecosystems, hence small changes in SOC due to N addition could significantly affect global climate change (Lehmann and Kleber, 2015). Recent studies suggest that separating soil C into POC and MOC enables an accurate prediction of SOC vulnerability to future climate change (Averill and Waring, 2018; Lavallee et al., 2020). Soil POC is formed mainly through plant litter fragmentation, largely made of a mixture of partially decomposed plant and decomposition by-products (Six et al., 2001), while soil MOC mainly comprises low molecular weight microbial compounds being bonded with soil minerals (Kleber et al., 2015). The chemical bonding of organic C and minerals makes MOC less susceptible to extracellular enzymes and decomposers, as a result, the higher fraction of MOC represents more stability of soil C (Fang et al., 2019; Lu et al., 2021). Therefore, assessing the response of soil C fractions under N addition is essential to accurately predict future soil C stability.

Several studies have reported that N addition effects on soil C stability are positive (Chen et al., 2020a; Lu et al., 2021), negative (Feng et al., 2022; Tang et al., 2023), and neutral (Ye et al., 2018; Feng et al., 2023). The heterogeneity of N addition duration, N fertilization types, and ecosystem types may contribute to the divergence (Li et al., 2021a; Xu et al., 2022). The traditional view is that POC is more sensitive to agricultural management practices (particularly N addition) which can serve as a short-term diagnostic indicator of changes in SOC (Cambardella and Elliott, 1992; Silveira et al., 2013; Plaza-Bonilla et al., 2014; Sheng et al., 2015). However, several studies have shown that changes in soil minerals and microbial physiology due to N short-term addition could cause great change in MOC (Chen et al., 2019; Xu et al., 2022). These comparative results suggested that the response of POC and MOC under different N
addition duration remain controversial. Furthermore, previous studies have shown that organic materials input greatly promoted SOC accumulation but reduced soil C stability (Du et al., 2020; Li et al., 2021a). By contrast, some studies found that the higher fraction of liable C and relatively narrower C: N of manure may increase soil C stability by facilitating the MOC accumulation (Samson et al., 2020). That is, the organic and inorganic N addition may have differential influences on the magnitudes and sizes of soil C fractions, thereby determining soil C stability. Hence, to improve the prediction of soil C stability, a broad-scale synthesis of the effect of N addition on soil C fractions is urgently needed.

Here, we synthesized 427 observations reporting the effect of N addition on POC and MOC pool globally. In general, POC was illustrated as a functional soil component for persistent SOC, and vulnerable to environmental change, hence we hypothesized that N addition would affect soil C stability by accruing more POC. Moreover, based on previous studies (Liu and Greaver, 2010; Liu et al., 2020; Xu et al., 2022), we predicted that the responses of soil C fractions to N addition depend on the N addition duration, fertilization types, and ecosystem types. The objective of our study was to reveal (a) the response of soil C fractions to N addition globally, and (b) to investigate the factors affecting the differential responses.

2. Materials and methods

2.1 Data collection

We used the Web of Science (http://apps.webofknowledge.com/) and China National Knowledge Infrastructure (www.cnki.net/) for an exhaustive search of articles published before September 2021 to explore the effects of N addition on soil C fractions. The keyword combinations used for research were (“particulate organic matter” OR “mineral associated matter” OR “particulate organic carbon” OR “mineral associated carbon”) AND (“N addition” OR “nitrogen addition” OR “N deposition” OR “nitrogen deposition” OR “nitrogen enrichment” OR “N enrichment” OR “fertiliz*”), without restriction on publication year. Articles to be included in our dataset need to meet the following criteria: (1) Considering that there are various methods of separation of soil C, the ones used in the meta-analysis had to be treated with sodium hexametaphosphate or NaI or sonication (75.3%, 11.5%, 13.2% of our database) to ensure complete soil separation. MOC was defined as heavier than 1.6–1.85 g/cm³ or smaller than 50–63µm, and POC in these articles was defined as lighter than 1.6–1.85 g/cm³ or larger than 50–63µm (Lavallee et al., 2020). (2) Articles must include accessible data for control and N addition treatments, and biotic and abiotic factors are similar between treatments. (3) The means, standard deviations (SDs), and sample sizes can be extracted or transformed from the articles; if SDs are unavailable, we use 10% of the mean instead. (4) Details of the nitrogen addition (e.g., N loading form, frequency, and experimental duration) are provided. (5) The most recent cases were used when the study involved multiple observations at different sampling times. We have drawn a PRISMA flow diagram to demonstrate the article selection process (Fig. S1). Finally, a total of 69 articles (51 of cropland, 16 of forest and 11 of grassland ecosystems) and 427 pairs of observations that met our criteria were used in our subsequent analysis (Fig. S2).
We extracted the POC and MOC contents from the 69 articles screened. If the articles further separated POC (fine POC and coarse POC), we identified their sum as POC. In addition, we defined the ratio of POC to SOC as the fraction of POC (fPOC); and the ratio of MOC to SOC as the fraction of MOC (fMOC), and they were used to characterize the stability of soil C (Fang et al., 2019; Chen et al., 2020b). For the study, all cases were from topsoil (0–30 cm). We recorded longitude, latitude, mean annual temperature (MAT), mean annual precipitation MAP, and ecosystem types (i.e., cropland, forest, and grassland) from the study. If climate data were incomplete, we obtained MAT and MAP from the WorldClim database (www.worldclim.org/). Also, we extracted the fertilization types of N addition (organic and/or inorganic N), frequency of N addition (times/year), and experimental duration (years). Specifically, the combinations of N additions included N, NP, NKP, M (manure), MN, MNP, and MNPK. Among them, treatments containing manure were considered as organic N additions, while urea, NH₄NO₃, NaNO₃, and NH₄Cl were treated as inorganic N additions. The frequency of N addition (<4, low level; ≥4, high level) and the duration of N addition (<5 short-term; ≥5 long-term) were also divided into different subgroups for comparison.

We recorded soil properties, microbial community, and extracellular enzymes from the article to explore the key modulators of N enrichment regulating changes in soil C fractions. Our datasets contain the following variables (Table. S1): (1) For soil properties, we extracted the soil pH, total soil nitrogen (TN), soil carbon to nitrogen ratio (C: N), total soil phosphorus (TP), soil bulk density (BD), soil water content (SWC), ammonium nitrogen (AN), and nitrate nitrogen (NN). (2) For the soil microbial community, we recorded soil microbial carbon (MBC), soil microbial nitrogen (MBN), fungal biomass (F), bacterial biomass (B), and total PLFA. (3) For soil extracellular enzymes, we recorded β-1,4-N-acetylglucosaminidase (NAG), β-glucosidase (BG), phenol oxidase (POX), and peroxidase (PER). Due to the limited number of samples, we, unfortunately, did not have sufficient data on plants (e.g., aboveground biomass (AGB) and belowground biomass (BGB), fine root biomass, etc.), and soil minerals (Ca²⁺, Mg²⁺, Al³⁺, Fe³⁺, etc.). Although these plant and soil mineral indicators have been shown to be closely related to changes in soil C fractions (Ye et al., 2018; Feng et al., 2022). When the above data is presented in graphical form, we use getdata2.2 to digitize the observation.

### 2.2 Statistical analysis

Firstly, We used natural log response ratio (ln RR) to assess the effect sizes of N addition (Hedges et al., 1999):

\[
\ln (RR) = \ln \left( \frac{X_N}{X_C} \right)
\]

where \( X_N \) and \( X_C \) are the mean values of the variable under N addition and control treatments, respectively. The variance (\( v \)) for each lnRR was calculated as follows:

\[
V = \frac{SD_Y^2}{n_MY^2} + \frac{SD_C^2}{n_CY^2}
\]
with \( n_C \) and \( n_N \) as the replicate numbers and \( SD_N \) and \( SD_C \) as the standard deviations of the variable (SD\(_S\)) for control and N addition treatments, respectively.

The mean weighted response ratio (RR\(_{++}\)) was calculated as:

\[
R_{++} = \frac{\sum_{i=1}^{m} \sum_{j=1}^{k} W_{ij} l_n R_{R_{ij}}}{\sum_{i=1}^{m} \sum_{j=1}^{k} W_{ij}},
\]

\[
W_{ij} = \frac{1}{v}
\]

with \( m \) as the group number, and \( k \) is the number of comparisons in the group. Also, RR\(_{++}\) is converted into percentages for better interpretation:

Percentage change (%) = \([\exp( RR_{++} ) - 1] \times 100\%

The effect of adding N variables is considered significant if the 95% confidence interval (CI) does not overlap with zero, and vice versa. The above calculations were all performed in MetaWin software (Sinauer Associates Inc., Sunderland, MA, USA).

Second, we plotted the frequency distribution of soil C fractions (POC, MOC, fPOC, fMOC, and SOC) (Fig. S3). Egger's test of publication using funnel function in R software (R 4.1.3) showed that there was no significant bias in the variables (Table. S2). Then, linear regression was used to test the relationships of the natural log response ratios of the soil C fractions with N addition duration and the response ratios of AN and MBC. These analyses were conducted using the R package "ggplot2". A model-selection analysis was conducted using the "glmulti" package of R software to examine the relative effects of multiple controlling factors on the SOC response to N addition (Zhou et al., 2020). The importance of each predictor was calculated as the sum of Akaike weights for all the models in which the variable was included, which can be identified as the overall support of each predictor across all models (Terrer et al., 2016). If the sum of Akaike weights of the variable exceeds the cutoff 0.8 then the variable is a critical predictor and vice versa (Calcagno and de Mazancourt, 2010). Since POC and MOC have completely different turnover times, we further analyzed the relative importance of soil C fractions and each variable on SOC accumulation at different N addition durations.

3. Results

3.1 Response of soil C fractions to N addition.

We found that N addition significantly increased SOC, POC, and MOC by 16.0%, 32.3%, and 8.8%, respectively (Fig. 1), but the fPOC and fMOC responses to N addition were diverse: fPOC was increased by 15.9%, while fMOC was declined by 6.3%, these indicate that N addition would reduce the soil C stability (Fig. 1).
3.2 Factors that drive the differential responses of soil C fractions to N addition.

For fertilization methods, the effect of organic N fertilization on soil C fractions was more significant than inorganic N fertilization ($p < 0.01$) (Fig. 2). Organic N fertilizer addition further contributed to the accumulation of SOC, POC, MOC, and fPOC, but further contributed to the reduction of fMOC (Fig. 2). Furthermore, N addition significantly altered the soil C fractions in cropland ecosystems, with minimal effects in natural ecosystems (Fig. 3). These responses in cropland ecosystem were mainly concentrated in POC. Besides, the N addition duration has various effects on soil C fractions ($p < 0.01$) (Fig. 3). Long-term N addition promotes both POC and MOC pool, while short-term N additions only significantly increased POC (Fig. 3). The model-selection analysis further showed that the response ratio of POC was the only predictor of SOC change under short-term N addition (Fig. 4b), while both the response ratio of POC and MOC were important predictors of SOC change under long-term N addition (Fig. 4c). Also, the response ratios of POC were significantly and positively correlated with the soil ammonium nitrogen (Fig. S4). In addition, the response ratio of MBC was significantly and positively correlated with the response ratio of MOC (Fig. S6).

4. Discussion

4.1 The effect of N addition on soil C fractions

Our meta-analysis revealed that N addition significantly increased soil POC (32.3%) and MOC (8.8%), suggesting N addition promotes the accumulation of the SOC pool, especially the active POC pool. Two possible explanations account for the results, first, we found a positive linear correlation between POC and ammonium nitrogen (Fig. S4), which suggests that the POC accumulation may be attributed to more N availability (Table. S1). Previous suggested that the rise in ammonium nitrogen can alleviate plant N deficiency, causing more plant biomass and structural plant components input thereby promoting POC formation (Liu et al., 2016; Schulte-Uebbing and de Vries, 2018; Ye et al., 2018). Second, N addition may increase MOC by inhibiting soil microbial oxidative enzyme activity (such as phenol oxidase) and reducing the decomposition of stable organic C by microorganisms (Chen et al., 2018) (Table. S1). Consistent with this deduction, Cusack et al. (2010) and Shen et al. (2018) attributed the enhanced MOC under N addition to decreases in oxidase. Notably, fPOC (15.9%) grew greatly while fMOC (-6.3%) declined dramatically with N addition, suggesting that SOC sequestration corresponded to a decrease in soil C stability. This may be due to the lower N cost and less protection of particulate organic matter (POM) compared to the mineral-associated organic matter (MOM) (Cotrufo et al., 2015; Cotrufo et al., 2019; Lavallee et al., 2020). Considering that N availability is one of the major limiting factors for soil C sequestration, the lower C: N ratio of POC gives it a greater potential to accumulate under N addition (Cotrufo et al., 2019). Also, compared to MOM, POM is none or protected by large aggregates, therefore it is more responsive to external N addition, which leads to an increase the soil C vulnerability (Hernandez-
Ramirez et al., 2009; Feng et al., 2022). Altogether, our research provides solid evidence to confirm that global N deposition will reduce soil C stability through changing soil C fractions.

4.2 The effects of N addition duration, N addition methods, and ecosystem types on soil C fractions

N addition effects on soil C fractions varied with N addition duration (Fig. 4a, Fig. S4). In the short-term (<5 years), N additions only contributed to the sequestration of SOC through increasing POC (Fig. 3, Fig. 4b), this indicates that the rapid growth of POC played a crucial role in the accumulation of SOC. The fundamental difference in the turnover times between POC and MOC could explain the distinct responses. Specifically, POC are vulnerable to microbial decomposition, have faster turnover times (years to decades), and therefore can respond quickly to N addition (Kleber et al., 2015; Lavallee et al., 2020).

Previous studies using $^{14}$C dating techniques have also found a short average residence time for POC (~2 years), further explaining that it can be rapidly preserved and favor SOC sequestration under N addition (Silveira et al., 2013; Plaza-Bonilla et al., 2014; Guo et al., 2022). By comparison with POC, MOC resists microbial decomposition by forming organic-mineral complexes or by occluding in micro-aggregates, resulting in higher stability and longer turnover times (decades to centuries) (von Lützow et al., 2007).

Thus, the MOC pool is relatively stable under short-term N addition. In addition, the results suggest that long-term (≥5 years) N addition contributes to SOC accumulation through both POC and MOC (Fig. 4c).

Previous studies illustrated that the higher microbial necromass plays a crucial role in regulating the sequestration of MOC under long-term N addition (Liang et al., 2017; Chen et al., 2020a). A significant enhancement in MBC was also observed under long-term N addition (Fig. S5). More microbial biomass grows, dies, and turns over to create necromass C, which causes more MOC formation through the in vivo pathway (Liang et al., 2017) (Fig. S7). Taken together, the divergent contributions of POC and MOC to SOC sequestration depend on N addition duration.

Consistent with our hypothesis, the response of soil C fractions to N addition is also influenced by vegetation types (Deng et al., 2020) (Fig. 2). N addition significantly altered soil C fractions in cropland, but not in natural ecosystems (forests and grasslands). (Fig. 3). Two possible reasons can be provided. First, cropland ecosystems tend to be relatively deficient in N and highly dependent on exogenous N inputs, as anthropogenic factors such as harvesting often eliminate plant-uptake N (Zhong et al., 2016). As a result, cropland under N addition tends to rapidly increase the soil active C fraction (POC) and lead to a decrease in soil C stability (Lou et al., 2011; Li et al., 2021b). Second, natural ecosystems, especially forests, tend to have large and frequent inputs of residue as a source of nutrients. Exogenous N inputs are smaller in comparison to the residue and therefore soil C fractions in natural ecosystems have a more stable response to N addition (Ren et al., 2017; Xu et al., 2022). This assertion is supported by Fang et al. (2019) and Yuan et al. (2020), who found that N addition in grasslands and forests had minimal effects on POC and MOC pools, possibly due to more plant inputs offset by faster decomposition or mineral protection.

Furthermore, we found that organic fertilizers were more capable than inorganic fertilizers in determining the changes in soil C fractions ($p < 0.001$) (Fig. 2). These could be ascribed to the following aspects.
Firstly, the application of organic fertilizers directly promotes the input of exogenous organic C and provides various effective nutrients for plant growth (Li et al., 2021a; Tian et al., 2022). More organic materials and plant biomass C are imported into the soil and fragmented to form more POC (Cotrufo et al., 2015). In addition, Organic materials in manure were considered a vital binding agent for soil aggregation, and it could promote the binding of soil primary particles and/or micro-aggregates, leading to the accumulation of higher macroaggregates and POC (Mustafa et al., 2020). Similarly, the results also showed that the application of organic fertilizer further promoted the accumulation of MOC (Fig. 2). This is partly because organic fertilizers increase microbial growth and carbon use efficiency (CUE). According to previous studies, organic N fertilizer could alleviate soil acidity, and facilitate microbial development to raise CUE, thereby improving soil MOC formation efficiency through the in vivo pathway (Fig. S4 and S5) (Liang et al., 2017; Xiao et al., 2021; Feng et al., 2022).

5. Conclusion

The elucidation of the divergent dynamics of soil C fractions under N addition offers a novel insight into the formation and stabilization of SOC (Fig. 5). We found that N addition significantly increased POC, MOC, and fPOC. The enhanced fraction of relatively active C pools illustrates the reduced global soil C stability, which may stimulate soil C emissions and strengthen C-climate feedback under future N deposition. Meanwhile, we provide global empirical evidence to support the traditional view that POC could predict the short-term SOC dynamics under N addition. Overall, findings highlight the importance of incorporating soil C fractions into Earth system models to better understand SOC dynamics under the context of increasing ecosystem N availability.

Declarations

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declaration of competing interest

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

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References


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Figures
Figure 1

**Effects of N addition on the soil C fractions.** Error bars represent 95% confidence intervals (CI); n = 427 in each variable. POC, particulate organic carbon; MOC, mineral-associated organic carbon; fPOC, the fraction of POC; fMOC, the fraction of MOC.
Figure 2

**Effects of N loading form on the soil C fractions.** The M indicates manure treatment; "×" indicates the combination of sub-treatment. The treatment containing manure is considered an addition of organic N and conversely, it is regarded as inorganic N addition. The p-values in the upper right corner represent between-group differences. Solid dots (●) represent changes in soil C fractions under N addition significantly influenced by the controlling factors (p < 0.05); hollow circles (○) indicate p > 0.05. The error bars represent 95% CI. The gray numbers on the left represent the sample size, and the vertical dashed lines represent the effect size of 0.

![Figure 2](image)

Figure 3

**Meta-analysis of average effect size (%) of soil carbon fractions on ecosystem types, N addition frequency, and duration.** Low N and high N frequency represent a low (<4) and high (≥4) N addition frequency (times/year), respectively; Short-term N addition and long-term N addition represent a short (<5) and high (≥5) experimental durations. Solid dots (●) represent changes in soil C fractions under N addition significantly influenced by the controlling factors (p < 0.05); hollow circles (○) indicate p > 0.05. The error bars represent 95% CI. The p-values in the upper right corner represent between-group differences. The gray numbers on the left represent the sample size, and the vertical dashed lines represent the effect size of 0.

![Figure 3](image)
Figure 4

Model-averaged importance of the predictors of N addition effects on SOC. We further analyzed the relative importance of soil C fractions and each variable on SOC accumulation at (b) short-term and (c) long-term N addition. A cutoff of 0.8 (the red dashed line) is set to differentiate between vital and nonessential predictors. The RR of POC, MOC, fPOC, and fMOC refers to the natural log response ratio of POC, MOC, fPOC, and fMOC, respectively. MAT, mean annual temperature; MAP, mean annual precipitation; Biome, different ecosystem types; Duration, N addition durations; Form, N loading methods.
Figure 5

Conceptual illustration of the dynamic response of soil C fractions to N addition. N addition significantly increased SOC pools but reduced soil C stability under different N addition duration, fertilization types, and cropland ecosystems, primarily through POC accrual. Specifically, under short-term N addition (< 5 years), the response ratio of POC was the only significant predictor of response to SOC accumulation; while under long-term N addition (≥ 5 years), the response ratio of MOC jointly drives SOC enhancement. The blue circles and yellow triangles represent newly formed POC and MOC under N addition, respectively. The percentage of POC and MOC in the whole square represents fPOC and fMOC in order, and the size of the whole square indicates the size of the SOC pool. The black borders around the squares represent variables (i.e. POC and/or MOC) that significantly affect the change in SOC. The red and blue arrows indicate the variables that are positively and negatively altered by N enrichment.

Supplementary Files

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