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RESEARCH ARTICLE

Depth-dependent responses of soil organic carbon under nitrogen deposition

Yuanliu Hu[1,2,3,4] | Qi Deng[1,2,3] | Thomas Kätterer[5] | Jørgen Eivind Olesen[4,6] | Samantha C. Ying[7] | Raúl Ochoa-Hueso[8,9] | Carsten W. Mueller[10,11] | Michael N. Weintraub[12] | Ji Chen[4,13,14]

[1]Key Laboratory of Vegetation Restoration and Management of Degraded Ecosystems/Guangdong Provincial Key Laboratory of Applied Botany, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou, China

[2]South China National Botanical Garden, Guangzhou, China

[3]University of Chinese Academy of Sciences, Beijing, China

[4]Department of Agroecology, Aarhus University, Tjele, Denmark

[5]Department of Ecology, Swedish University of Agricultural Sciences, Uppsala, Sweden

[6]Aarhus University Centre for Circular Bioeconomy, Aarhus University, Tjele, Denmark

[7]Department of Environmental Sciences, University of California, Riverside, California, USA

[8]Department of Biology, IVAGRO, University of Cádiz, Campus de Excelencia Internacional Agroalimentario (CeiA3), Cádiz, Spain

[9]Department of Terrestrial Ecology, Netherlands Institute of Ecology (NIOO-KNAW), Wageningen, The Netherlands

[10]Institute of Ecology, Chair of Soil Science, Technische Universitaet Berlin, Berlin, Germany

[11]Department of Geosciences and Natural Resource Management, University of Copenhagen, Copenhagen, Denmark

[12]Department of Environmental Sciences, University of Toledo, Toledo, Ohio, USA

[13]State Key Laboratory of Loess and Quaternary Geology, Institute of Earth Environment, Chinese Academy of Sciences, Xi'an, China

[14]Institute of Global Environmental Change, Department of Earth and Environmental Science, School of Human Settlements and Civil Engineering, Xi'an Jiaotong University, Xi'an, Shaanxi Province, China

Correspondence

Ji Chen, State Key Laboratory of Loess and Quaternary Geology, Institute of Earth Environment, Chinese Academy of Sciences, Xi'an 710001, China.
Email: ji.chen@agro.au.dk

Qi Deng, Key Laboratory of Vegetation Restoration and Management of Degraded Ecosystems, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou, Guangdong 510650, China.
Email: dengqi@scbg.ac.cn

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Abstract

Emerging evidence points out that the responses of soil organic carbon (SOC) to nitrogen (N) addition differ along the soil profile, highlighting the importance of synthesizing results from different soil layers. Here, using a global meta-analysis, we found that N addition significantly enhanced topsoil (0–30 cm) SOC by 3.7% (±1.4%) in forests and grasslands. In contrast, SOC in the subsoil (30–100 cm) initially increased with N addition but decreased over time. The model selection analysis revealed that experimental duration and vegetation type are among the most important predictors across a wide range of climatic, environmental, and edaphic variables. The contrasting responses of SOC to N addition indicate the importance of considering deep soil layers, particularly for long-term continuous N deposition. Finally, the lack of depth-dependent SOC responses to N addition in experimental and modeling frameworks has likely resulted in the overestimation of changes in SOC storage under enhanced N deposition.

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KEYWORDS
carbon cycle–climate feedbacks, carbon losses, nitrogen addition duration, soil carbon sequestration, soil profiles

1 | INTRODUCTION

Excessive nitrogen (N) deposition from human activities has continuously increased over the past decades (Fowler et al., 2013; Gruber & Galloway, 2008; IPCC, 2021), with substantial consequences on soil organic carbon (SOC) dynamics (Liu & Greaver, 2010; Pregitzer et al., 2008; Xu et al., 2021). Despite the rapid increase in the number of studies reporting the effects of N addition on SOC, no consensus has been reached on the response along the soil profile. For example, Bowden et al. (2019) found that N addition significantly increased SOC in the topsoil in boreal forests (0–30 cm), whereas N addition significantly decreased SOC in temperate grasslands when the whole soil profile (0–100 cm) was considered (Li et al., 2014; Poeplau et al., 2018). Thus, a better understanding of N deposition impacts on SOC at different depths is urgently needed to accurately predict carbon (C) cycle–climate feedbacks.

The topsoil typically receives the majority of plant-derived C inputs, and it thus has the highest SOC and microbial biomass content (Cochran et al., 1989). It is proposed that topsoil SOC displays a relatively high decomposition rate and a rapid exchange of C with the atmosphere (Hartemink et al., 2020; Rumpel & Kögel-Knabner, 2011). In contrast, SOC buried in the subsoil is generally assumed to be more persistent, with a longer turnover time (Hicks Pries et al., 2017). Based on this assumption, the Intergovernmental Panel on Climate Change has primarily emphasized the 0–30 cm soil layer in the greenhouse gas inventory (IPCC, 2006, 2021). Indeed, this sampling depth has been adopted by many experiments and models when investigating SOC dynamics under various conditions. However, the assumption of the persistence of SOC in the subsoil has recently been challenged (Chen et al., 2023; Li et al., 2022; Luo et al., 2019). Several studies have demonstrated the depth-dependent responses of SOC to nutrient addition (Fierer et al., 2003), climate warming (Wang et al., 2022), and land use change (Chen et al., 2022; Mobley et al., 2015). Furthermore, long-term regional and global studies have revealed different SOC accumulation rates and driving forces when comparing topsoil to subsoil (Balesdent et al., 2018; He et al., 2022; Li et al., 2022; Sun et al., 2023). These results imply that a lack of exploration for subsoil SOC may hamper our ability to assess the potential of soil C sequestration under future climate change scenarios.

Despite the growing recognition of the importance in subsoil SOC, only a limited number of studies have investigated the effects of N addition on SOC in subsoil across the globe. Several recent meta-analyses indicated that the effects of N addition on SOC were highly dependent on soil and microbial properties (Ni et al., 2022; Niu et al., 2021), with both types of variables showing substantial variations along soil profiles (Eilers et al., 2012; Federle et al., 1986; Mathieu et al., 2015). Other studies further suggested that the response of SOC to N addition may not always be aligned in the topsoil and subsoil, and in some cases, responses may even be contradictory. For example, Canary et al. (2000) and Li et al. (2014) found that N addition significantly increased SOC in the topsoil, whereas SOC decreased in the subsoil, leading to neutral or even negative responses of SOC to N addition when the whole soil profile was considered. These results demonstrate that neglecting the effects of N addition on subsoil SOC may lead to biased interpretations, or even misleading conclusions about the potential consequences of increased N deposition on SOC sequestration.

In this study, we conducted a comprehensive meta-analysis to examine the effects of N addition on SOC in both topsoil and subsoil. We compiled data from 177 N addition experiments conducted worldwide, regarding 0–30 cm as topsoil and 30–100 cm as subsoil according to the recommendations from IPCC (IPCC, 2021; Minasny et al., 2017). We also recorded information on environmental factors (mean annual temperature and precipitation) and experimental protocols (N addition form, rate, frequency, and duration) that may be relevant in determining the responses of SOC to N addition in both topsoil and subsoil. Given that N addition effects on SOC may be cumulative, we also distinguished the responses of SOC to N addition between short-term (<5 years) and long-term (≥5 years) studies. Overall, we addressed two specific questions in this study: (1) How did SOC in topsoil and subsoil respond to N addition at the global scale? (2) What were the important modulators of the responses of SOC to N addition in topsoil and subsoil?

2 | MATERIALS AND METHODS

2.1 | Data collection

We searched for peer-reviewed articles published before December 2023 from Web of Science (http://apps.webofknowledge.com/), Google Scholar (http://scholar.google.com/), and China National

Knowledge Infrastructure (http://www.cnki.net/). In order to find articles reporting the effects of N addition on SOC from different soil depths, the used keywords or combinations of keywords were: (a) "nitrogen addition" or "nitrogen enrichment" or "nitrogen fertilizer" or "nitrogen elevated" or "nitrogen deposition" and (b) "soil organic carbon" or "soil organic matter" and (c) "soil depth" or "soil layer" and (d) "terrestrial" or "soil" or "land."

To explore the N-induced changes in SOC across different soil layers, observations were removed according to the following criteria (Figure S1): (1) Measurements from croplands and other managed ecosystems, because disturbance through agricultural practices may alter the response of SOC to N addition along soil depth; (2) studies did not clearly specify soil sampling depth; (3) studies where other nutrients were added (e.g., P, K, Ca, compost, or slurry additions) or other treatments were imposed (e.g., CO_2, warming, or precipitation change); (4) missing details on N addition methods (fertilization rate, frequency, form, and duration); (5) N addition studies without directly applying N to the soil. Following the common soil sampling method, we did not consider the response of SOC to N addition in the litter layer in this study. Finally, we synthesized data (overview is provided in Data S1) on the responses of SOC to N addition from 177 published articles (Figure S2).

We recorded SOC concentration (percentage of SOC per soil mass) in the corresponding treatments with and without N addition of each study involved in this dataset, respectively. Overall, 8.5% of the observations in this dataset reported SOC storage representing mass C per volume, which could not be converted to SOC concentration on a mass basis because of unreported soil bulk density. To identify the influence of changes in soil bulk density on the responses of SOC to long-term N addition (Chen et al., 2019), we also recorded soil bulk density with and without N addition. The soil sampling depth in this dataset ranged from 5 to 100 cm, with an average depth of 25 cm. To be consistent with the IPCC's soil layer classification method, data from different soil layers were categorized into 0–30 cm (topsoil) and 30–100 cm (subsoil).

Furthermore, to explore the underlying mechanisms related to the responses of SOC to N addition, we also recorded vegetation type (grassland and forest), mean annual temperature (MAT), mean annual precipitation (MAP), longitude, latitude, background N deposition rate (BND), and N addition method (experimental duration, added N rate, the ratio of BND to added N rate and added N frequency) for each experimental site. The experimental duration of N addition studies included in our analysis ranged from 1 to 58 years. The cutoff of 5 years for short-term versus Longer term studies was chosen based on a large survey of long-term research in ecology and evolution, indicating that experiments lasting less than 5 years often quantified only the immediate and transient effects of perturbation (Kuebbing et al., 2018). Most studies added N in each year along the experimental duration, except for the study conducted by Canary et al. (2000), in which N was added intermittently every few years. In this dataset, the N addition rate ranged from 0.5 to 64 $g\,m^{-2}\,year^{-1}$. As N application forms, we considered inorganic N (NH_4, NO_3, or NH_4NO_3), organic N (urea), and the mixture of inorganic and organic N (NH_4, NO_3, and urea). Besides, we obtained unreported MAT and MAP from the WorldClim database (Fick & Hijmans, 2017), which resulted in the inclusion of a broad range of MAT (−4.6 to 26°C) and MAP (69–4300 mm $year^{-1}$) in our dataset. Unreported background N deposition rate was collected from the Global N deposition database (ORNL DAAC, 2017). The missing standard deviation was imputed using Rubin and Schenker's resampling approach from studies with similar means (Rubin & Schenker, 1991). When results were presented graphically, we used GetData Graph Digitizer v.2.24 (http://getdata-graph-digitizer.com/) to digitize the data.

2.2 | Data analysis

We used the natural log response ratio to assess the effect of N addition on each variable. Each individual observation was weighted by the inverse of the mixed model variance, ensuring that meta-analysis data statistics have simpler standard sampling distributions while avoiding the possibility of unequal variance effects on statistical results (Hedges et al., 1999).

The natural log response ratio (ln R) was calculated as:

$$\ln R = \ln\left(X_N / X_C\right)$$

where X_C and X_N are the mean values of the studied variables in the control and N addition treatments, respectively.

The variance (V) of the logarithmic effect size was calculated as:

$$V = S_N^2 / \left(n_N X_N^2\right) + S_C^2 / \left(n_C X_C^2\right)$$

where S_C and S_N are the standard deviation in the control and N addition treatments, respectively, and n_C and n_N are the number of replicates in the control and N addition treatments, respectively.

A mixed-effects model was used to determine the effect of N addition on the selected variables through "rma.mv" function from the R package "metafor" (Viechtbauer, 2010). We considered the "Publication" as random effect in the mixed-effects model, which would ensure the independence of each observation (Chen et al., 2018). The effects of N addition were considered significant if the 95% confidence intervals did not overlap with zero. The results were reported as mass percentage change with N addition to ease interpretation.

We carried out a model selection analysis to determine which were the main environmental and procedural variables driving the response of SOC to N across depths. Model selection was based on the corrected Akaike information criterion (AIC corrected for small samples). A predictor including models with large Akaike weights was assigned with a high importance value. Thus, the cumulative Akaike weight of the models containing the specific predictor was used to indicate the relative importance value for this predictor. A cutoff value of 0.8 was set to identify the critical and non-critical predictors (Terrer et al., 2016; van Groenigen et al., 2011). This analysis was conducted through the “glmulti” package in R (Calcagno & De Mazancourt, 2010).

3 | RESULTS

Averaged across all studies, N addition significantly increased SOC in the topsoil by 3.7% (95% CIs, 2.2–5.1%, $p<.001$). However, N addition did not affect subsoil SOC (Figure 1a). The depth-dependent responses of SOC to N addition were consistent regardless of vegetation type (forest and grassland; Figure 1b). Based on the corrected Akaike information criterion, the responses of SOC to N addition were best predicted by soil layer and experimental duration (Figure S3). The results from mixed linear regression model showed a significant interactive effect of soil layer and experimental duration on the responses of SOC to N addition (Table S1).

In the topsoil, experimental duration and vegetation type were the important predictors of the response of SOC to N addition (Figure 2a). Short-term (<5 years) N addition did not change topsoil SOC (−0.6% to 3.3%, $p=.18$; Figure 3a), whereas long-term (≥5 years) N addition significantly increased topsoil SOC by 5.6% (3.7%–7.5%, $p<.001$). The time-dependent responses of topsoil SOC to N addition were consistent regardless of vegetation type (Figure S4).

Regarding the subsoil, the responses of SOC to N addition were best predicted by experimental duration (Figure 2b). Short-term (<5 years) N addition significantly increased SOC by 4.3% (1.1%–7.6%, $p<.05$; Figure 3b), whereas long-term (≥5 years) N addition decreased SOC by −3.9% (−1.4% to −6.3%, $p<.01$). The significant decreases in subsoil SOC under long-term N addition were only observed in forests; however, there was a lack of significant interactive effects of vegetation type and experimental duration on the responses of subsoil SOC to N addition (Table S1; Figure S4). Additionally, there was a positive relationship between the responses of topsoil and subsoil SOC to N addition in the short-term N addition studies, whereas no significant relationship was found between the responses of topsoil and subsoil SOC to N addition in the long-term studies (Figure 3c).

4 | DISCUSSION

4.1 | Nitrogen addition increased topsoil organic carbon over time

We found an increase in topsoil SOC under N addition (Figure 1a). Increased N availability under N addition likely stimulates plant growth and litter inputs (Greaver et al., 2016; Reay et al., 2008). The additional litter inputs can be partly incorporated into soils, contributing to enhanced topsoil SOC, as demonstrated in several N addition studies (Canary et al., 2000; Hyvönen et al., 2008; Liao et al., 2023). Nitrogen addition may also enhance topsoil SOC by repressing soil C losses (Janssens et al., 2010; Lu et al., 2021). For example, N addition often causes soil acidification or an increase in osmotic stress, potentially suppressing microbial growth and activity, and consequently reducing C losses from microbial respiration (Treseder, 2008; Zhou et al., 2014). The N-induced plant growth may also lead to increased uptake of base cations, thereby accelerating soil acidification and inhibiting microbial activity (Duan et al., 2004). Similarly, N addition may inhibit the phenol oxidase activity of white-rod basidiomycetous fungi in the floor of hardwood forests, leading to a greater accumulation of under-decomposed litter (Chen et al., 2018; Waldrop et al., 2004). In addition, the potential reduction in soil C losses under N addition may also be related to the enhanced stabilization of SOC compounds that are typically subject to physical and chemical shielding (Figure S5; Jilling et al., 2021; Treseder, 2004).

Our study indicated that the increased topsoil SOC only manifested with long-term N addition (Figure 3a), which was supported by recent meta-analyses (Lu et al., 2023; Xu et al., 2021). First, this

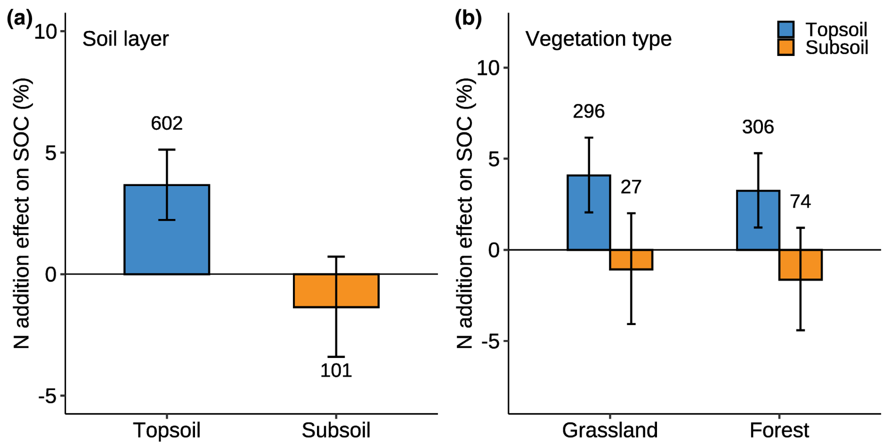

FIGURE 1 Nitrogen (N) addition only increased soil organic carbon (SOC) in the topsoil but not in the subsoil. (a) Meta-analysis of the effects of N addition on SOC when grouped by soil layers. (b) Meta-analysis of the effects of N addition on SOC when grouped by soil layers and vegetation types. Error bars represented bootstrap 95% confidence intervals (CIs). The effects of N addition were considered significant if the 95% CIs did not overlap with zero; the numbers above or below the error bars indicated sample sizes.

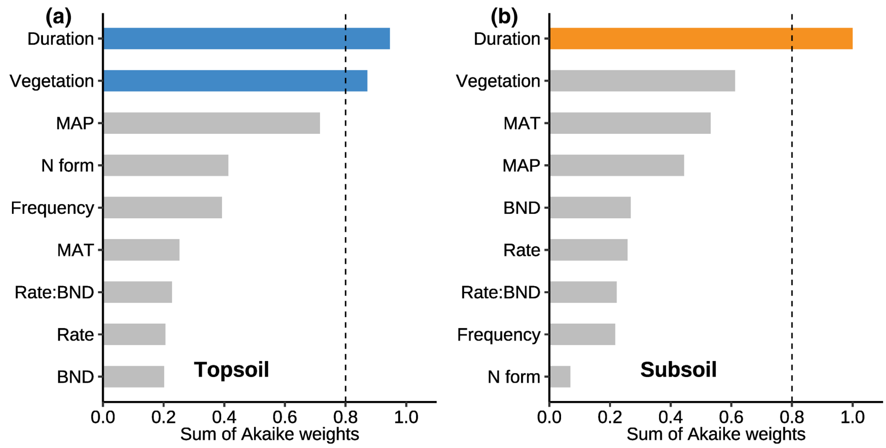

FIGURE 2 Experimental duration was the most important predictor in the responses of soil organic carbon (SOC) to nitrogen (N) addition in both topsoil and subsoil. (a) Model selection analysis identified that experimental duration and vegetation type were the important predictors of the response of SOC to N addition in the topsoil; the dashed line indicated the cutoff to distinguish important predictors that exceeded the 0.8 sum-of-Akaike-weights threshold. (b) Model selection analysis identified that experimental duration was the important predictor of the response of SOC to N addition in the subsoil. BND, background N deposition rates; duration, experimental duration; frequency, N addition frequency; MAP, mean annual precipitation; MAT, mean annual temperature; rate, N addition rate.

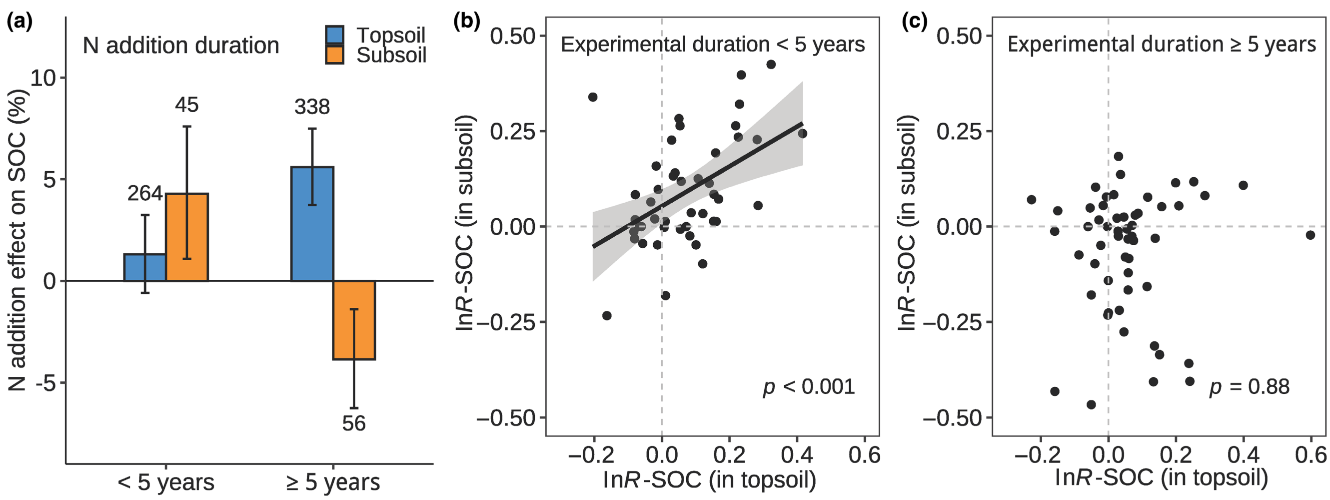

FIGURE 3 The effects of nitrogen (N) addition on soil organic carbon (SOC) depend on experimental duration. (a) In the topsoil, short-term N addition (<5 years) had nonsignificant effect on SOC, whereas long-term N addition (≥5 years) significantly increased SOC. In the subsoil, short-term N addition significantly increased SOC, whereas long-term N addition decreased SOC; error bars represented bootstrap 95% confidence intervals (CIs); the effects of N addition were considered significant if the 95% CIs did not overlap with zero; the numbers above or below the error bars indicated sample sizes. (b) In the short-term N addition studies, there was a significant positive relationship between the responses of SOC (ln*R*-SOC) in the topsoil and in the subsoil [coefficient of determination (r^2) = .23, p < .001]; the light gray area indicated the confidence interval around the regression line. (c) In the long-term studies, the relationship between the responses of SOC in the topsoil and the subsoil was not significant (r^2 = .01, p = .88).

result may suggest that it takes time for SOC accumulation to be observable. For example, Xu et al. (2021) suggested that significant increases in plant C inputs and topsoil SOC were typically observed in N addition studies lasting more than 3 years. Some studies also indicated that the plant C inputs at the initial stage of N addition may not cause as rapid SOC storage as previously assumed (Lu et al., 2023; Vourlitis & Hentz, 2016). However, a recent study based on global grasslands showed that SOC increased significantly under short-term N addition (<5 years), probably due to the inclusion of both topsoil and subsoil SOC in the database (Liu et al., 2023). Alternatively, the time-dependent response of topsoil SOC to N addition may be linked to the balance between plant C inputs and soil C losses (Janssens et al., 2010; Pregitzer et al., 2008; Zhou et al., 2014). For example, in the initial stage of N addition, alleviation of microbial N limitation may be associated with stimulated microbial respiration and C losses, which may offset the positive effects of plant C inputs on topsoil SOC, especially in N-poor ecosystems (Figure S6; Ågren et al., 2001; Lu et al., 2011). In the later stage of N addition, microbial activities may be repressed by N-induced soil acidification or increased osmotic stress (Zhou et al., 2014). Suppressed microbial activities and associated C losses have indeed been found to be significant under long-term N addition (Ning et al., 2021; Treseder, 2008).

4.2 | Long-term N addition decreased subsoil organic carbon

In contrast to the N-induced increases in topsoil SOC, we found that subsoil SOC significantly increased in short-term N addition studies (<5 years) but decreased in the long-term studies (≥5 years; Figure 3a). One possible reason for the increased subsoil SOC under short-term N addition may be attributed to the increased C leaching from aboveground litter decomposition (Li et al., 2021; Sinsabaugh et al., 2004). For example, the increased C leaching was likely associated with accelerated decomposition of N-induced additional litter inputs in the topsoil under short-term N addition (Wang et al., 2019). In the early stages of N addition, aboveground C input is typically associated with less efficient decomposition and lower C stabilization, which may stimulate the leaching of dissolved C downward to the subsoil, resulting in increased subsoil SOC (Pregitzer et al., 2004). In contrast to the topsoil, increased N availability within short-term N addition may not stimulate subsoil SOC mineralization and associated C losses due to the lack of fresh C and energy supply in this soil layer (Figure S6; Wang et al., 2014). Additionally, the positive effects of increased plant C inputs on subsoil SOC in the short term may also be related to the inherently lower SOC in this layer (Balesdent et al., 2018).

Why did long-term N addition significantly decrease subsoil SOC? This unexpected result can be partly attributed to the potential reduction in the downward movement of C along the soil profile in the later stage of N addition. For example, long-term N addition may repress litter decomposition, which likely results in accumulation of fresh aboveground litter in the humus layer instead of being transported into deeper soil layers (Franklin et al., 2003; Treseder, 2004). A 10-year N addition study conducted in northern American broadleaved forests revealed that the accumulation of SOC derived from leaf litter was observed only in the topsoil (Pregitzer et al., 2008). Besides, in the long term, the alleviated microbial N limitation with N addition is generally associated with decreased oxidase activities and increased formation of stable SOC in the topsoil (Chen et al., 2018; Lu et al., 2021), thereby reducing the C leaching along the soil profile (Kaiser & Kalbitz, 2012). This explanation is supported by both increased particulate and mineral-associated organic carbon in the topsoil only observed in long-term N addition studies (Figure S5; Qi et al., 2023). Similarly, bioturbation contributes to mixing SOC from the topsoil to the subsoil (Wilkinson et al., 2009), whereas this process may be repressed in the later stage of N addition due to possible soil acidification or toxicity effects on soil organisms (Jansone et al., 2020).

There was a lack of a significant positive correlation between the responses of SOC in the topsoil and the subsoil to long-term (as opposed to short-term) N addition (Figure 3c). This is likely attributed to contrasting mechanisms driving the responses of SOC to N addition in shallow versus deeper layers. In contrast to the topsoil, the subsoil may become more susceptible to losing C through microbial-mediated SOC decomposition in the later stage of N addition (Fontaine et al., 2007; Karhu et al., 2016). For example, long-term N addition may stimulate the decomposition of mineral-associated SOC in the subsoil as indicated by increases in microbial biomass (Figure S6), while this process is generally suppressed by insufficient energy supply within this soil layer compared to the topsoil (Henneron et al., 2022; Jilling et al., 2021). This explanation could be attributed to more energy investment from plants in the form of root exudates for resource acquisition, such as phosphorus and water, as the demand of plants for these resources gradually increases with the continuous N addition (Li et al., 2016; Peñuelas et al., 2013). For example, N addition significantly increased fine root biomass in the subsoil after 5 years (Yan et al., 2017), but decreased it in the first year of N addition (Zhu et al., 2021). Similarly, more C investment from plants in root growth for resource uptake in the later stage of N addition may induce priming effects of SOC decomposition. This N-induced root exploration for resource acquisition may also increase the accessibility of subsoil SOC that is often persistent due to physical separation from decomposers (Henneron et al., 2022; Salomé et al., 2010). The C losses from the decomposition of recalcitrant SOC may outweigh the potential increases in root C inputs (Mobley et al., 2015; Shahzad et al., 2019). Additionally, a recent 20-year study even found that N addition could accelerate the decomposition of ancient SOC in the subsoil by mitigating the oxygen limitation of SOC mineralization through the accumulation of nitrate (Qin et al., 2023), as nitrate is an alternative electron accepter for microbial respiration (Sierra & Renault, 1998).

4.3 | Implications

Understanding the depth-dependent responses of SOC to N addition can help us reconcile the apparently conflicting results often reported from individual studies (Deng et al., 2020; Xu et al., 2021; Zheng et al., 2022). It is widely recognized that responses of SOC to N addition are often cumulative, which takes time to be statistically significant. This meta-analysis study underlines the importance of considering soil depth together with time, as we found the contrasting responses of topsoil and subsoil SOC to short- and long-term N additions. Our finds thus imply that both topsoil and subsoil layers need to be evaluated for a more realistic understanding of the responses of SOC to N addition, especially in the context of long-term field experiments. Importantly, the sharp decline in subsoil SOC in response to long-term N addition presented here may challenge the previously assumed role of soils as a global SOC sink under increased N deposition (Janssens et al., 2010; Lu et al., 2011; Nave et al., 2009; Xu et al., 2021). However, most current N addition studies have primarily focused on the responses of SOC in the topsoil (typically in 0–20 cm or 0–30 cm), which may lead to biased conclusions regarding SOC sequestration. In addition, to be consistent with the IPCC's soil layer classification method, some observations of SOC in the soil layers of 20–40 cm or 20–60 cm were considered as subsoil SOC in this study (Figure S7). Considering the crucial role of subsoils for SOC sequestration (Jobbágy & Jackson, 2000; Rumpel & Kögel-Knabner, 2011; Shi et al., 2020), our study highlights the necessity to distinguish the responses of SOC to increased N deposition across different soil layers, rather than typically extrapolating the responses of SOC to subsoil based on its responses in the topsoil. Overall, our findings suggest the overestimation of soil C sequestration under N addition if without considering the subsoil C dynamics, highlighting the necessity for explicit incorporation of depth-dependent responses of SOC into current global C cycle models.

AUTHOR CONTRIBUTIONS

Yuanliu Hu: Data curation; formal analysis; writing – original draft. **Qi Deng:** Conceptualization; data curation; writing – original draft. **Thomas Kätterer:** Data curation; writing – review and editing. **Jørgen Eivind Olesen:** Data curation; writing – review and editing. **Samantha C. Ying:** Data curation; writing – review and editing. **Raúl Ochoa-Hueso:** Data curation; writing – review and editing. **Carsten W. Mueller:** Data curation; writing – review and editing. **Michael N. Weintraub:** Data curation; writing – review and editing. **Ji Chen:** Conceptualization; data curation; writing – original draft.

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CONFLICT OF INTEREST STATEMENT

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 article.

DATA AVAILABILITY STATEMENT

The data associated with this paper are available from figshare (https://doi.org/10.6084/m9.figshare.25358857.v1).

ORCID

Yuanliu Hu https://orcid.org/0000-0003-4137-225X
Qi Deng https://orcid.org/0000-0001-8208-9763
Thomas Kätterer https://orcid.org/0000-0002-1751-007X
Jørgen Eivind Olesen https://orcid.org/0000-0002-6639-1273
Samantha C. Ying https://orcid.org/0000-0002-1247-2529
Raúl Ochoa-Hueso https://orcid.org/0000-0002-1839-6926
Carsten W. Mueller https://orcid.org/0000-0003-4119-0544
Michael N. Weintraub https://orcid.org/0000-0002-9623-2855
Ji Chen https://orcid.org/0000-0001-7026-6312

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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