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RESEARCH ARTICLE

# Nitrogen stimulation of soil carbon content differs between two types of mycorrhizal-dominated ecosystems

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## Abstract

1. Increasing nitrogen (N) deposition has been suggested to favour arbuscular mycorrhizal (AM) plants over ectomycorrhizal (ECM) plants, but how mycorrhizal association mediates N impacts on soil organic carbon (SOC) remains unclear.
2. By synthesizing 315 N addition studies globally, we investigated the effects of N addition on SOC content in ecosystems dominated by ECM and AM plants (ECM and AM ecosystems).
3. Our results showed that SOC increased by 4.1% in ECM ecosystems, likely due to reduced ligninase activity. In AM ecosystems, SOC did not change during the first 5 years of N addition, but increased by 7.3% over long-term N addition (≥5 years), driven by increased litter inputs and progressive soil acidification.
4. These findings highlight that N addition enhances SOC in both ECM and AM ecosystems via distinct mechanisms and temporal dynamics, implying that the potential of soil C sequestration may differ between the two ecosystems from short- to long-term N addition.

**KEYWORDS**
carbon-degrading enzymes, climate change, mycorrhizal association, nitrogen acquisition strategies, plant–microbe interaction

## 1 | INTRODUCTION

Global atmospheric nitrogen (N) deposition has increased greatly over the last decades, with significant consequences for terrestrial carbon (C) sequestration (IPCC, 2021; Tang et al., 2023; Yang et al., 2025). In terrestrial ecosystems, over 80% of plant species associate with either ectomycorrhizal (ECM) fungi or arbuscular mycorrhizal (AM) fungi, representing distinct N-acquisition strategies (Phillips et al., 2013; Smith & Read, 2010). As such, mycorrhizal association has been identified as the primary control of the N fertilization effect, with stimulation of AM

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plant growth but no or even negative effect on the ECM plant growth (Averill et al., 2018; Jo et al., 2019). Different mycorrhizal associations are also expected to regulate both litter inputs and microbially mediated soil C decomposition, thus affecting soil C content. Although several studies have identified that the dominant mycorrhizal type in an ecosystem regulates the responses of soil C processes to N addition, how these regulations affect soil C content remains controversial (Hu et al., 2024; Yang et al., 2023; Zhang et al., 2025). Given that soil stores three to four times more C than vegetation (Lehmann & Kleber, 2015; Schmidt et al., 2011), a comprehensive assessment of mycorrhizal types on the response of soil C content to N addition is critically important for the present and future terrestrial C sink.

The mechanisms by which N addition affects soil C processes differ fundamentally between ecosystems dominated by AM and by ECM plants (AM and ECM ecosystems) and are considerably more complex than its effects on plant growth. For example, the generally stronger growth response of AM plants to N addition is expected to increase litter inputs and, consequently, soil C content relative to ECM ecosystems (Chen et al., 2025). However, enhanced plant productivity in AM plants may also stimulate microbial decomposition through priming effects, particularly when N addition exacerbates microbial C or phosphorus (P) limitation (Waldrop et al., 2004). In contrast, increasing evidence suggests that N addition may enhance soil C content in ECM ecosystems by alleviating plant N limitation and reducing ECM fungi activities (Argiroff et al., 2022; Zak et al., 2019). Specifically, N addition may suppress the production of extracellular enzymes, such as ligninase, that catalyse the decomposition of complex organic matter, thereby slowing soil organic matter turnover and promoting soil C accumulation. These contrasting mechanisms suggest that the response of soil C content to N addition may differ between AM and ECM ecosystems, highlighting the need to quantify these differences for accurately predicting how N deposition affects terrestrial soil C sinks.

Given that emerging N addition experiments conducted over long-term (≥5 years), several studies reveal a time-dependent response of soil C content (Lu et al., 2023; Pregitzer et al., 2008; Xu et al., 2021). These studies found that the N-enhanced soil C content often becomes significant in the later experimental stage, owing to the cumulative increase in litter inputs, whereas this resulting C gain effect may initially be offset by N-stimulated microbial decomposition. This temporal dynamic may be more evident in AM than in ECM ecosystems, as AM plants rely largely on free-living microbes for N acquisition, making decomposition may be more prone to priming under microbial C and phosphorus (P) limitation in the early stage, but suppressed as soil acidification intensifies later (Midgley & Phillips, 2014; Xu et al., 2021). In contrast, ECM fungi differ fundamentally from AM fungi in their ability to produce lignin-degrading enzymes. Because ligninase production is highly sensitive to soil N availability, N addition may rapidly suppress ligninase, thereby reducing the decomposition of complex organic matter and enhancing soil C content even in the short term (Waldrop et al., 2004). These differential sensitivities of soil C processes to N addition duration between AM and ECM ecosystems imply that failing to integrate more extensive long-term N addition studies may lead to a misrepresentation of the soil C content responses of the two ecosystems.

Here, we conducted a comprehensive meta-analysis of 997 observations from 315 peer-reviewed articles, including both short-term (<5 years) and long-term (≥5 years) studies (Figure S1), to investigate the effects of N addition on soil organic carbon (SOC) content in AM vs. ECM ecosystems. We hypothesized that: (1) SOC content in ECM ecosystems would rapidly increase even under short-term N addition, due to alleviation of plant–microbe competition for N and suppression of ECM fungal decomposition activities; and (2) SOC in AM ecosystems would show a minor response to short-term N addition but increase significantly under long-term N addition as a result of progressively greater litter inputs and increasing soil acidification and microbial nutrient limitation.

## 2 | MATERIALS AND METHODS

### 2.1 | Data collection

We searched for peer-reviewed articles published prior to May 2026 using the following publicly accessible databases: Web of Science (http://apps.webofknowledge.com/), Google Scholar (http://scholar.google.com/) and China National Knowledge Infrastructure (http://www.cnki.net/). In this study, the following Boolean search strings were used as follows: ("nitrogen deposition" OR "nitrogen addition" OR "nitrogen enrichment" OR "nitrogen fertilizer" OR "nitrogen elevated") AND ("soil carbon" OR "soil organic carbon" OR "soil organic matter") AND ("soil" OR "terrestrial" OR "land").

We excluded studies based on several criteria. (1) In order to explore the changes of SOC under N addition in AM and ECM ecosystems, we excluded studies that did not report mean value and sample size of SOC in the control or N addition treatments. (2) We also excluded studies that dominant mycorrhizal type could not be clearly identified at the experimental site. (3) Because litter input and microbial C cycling may be altered by agricultural practices, we excluded studies from croplands or other managed ecosystems. (4) We excluded studies that lacked the information on N addition methods (e.g. N addition rate, experimental duration, N addition frequency and form). (5) We excluded studies where other treatments were imposed (e.g. warming, elevated carbon dioxide, salinization or precipitation change) or other nutrients were added (e.g. P, K, Ca or compost). (6) We excluded studies not conducted in the field, such as greenhouse, potted-plant, growth-chamber and laboratory experiments. Totally, we synthesized data from 315 published articles (Text S1).

We identified mycorrhizal type (AM vs. ECM ecosystems) at each experimental site, according to the reported dominant plant species at the sites (Terrer et al., 2021). Specifically, an experimental site was classified as an AM or ECM ecosystem if it met at least one of the following criteria: (1) the site consisted of a pure or near-pure stand of tree species associated with a single mycorrhizal type; (2) dominant tree species accounted for >65% of total basal area, above-ground biomass or similar measures, and

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these dominant species belonged to the same mycorrhizal type; or (3) all dominant or co-dominant tree species belonged to the same mycorrhizal type and were explicitly described as 'absolutely dominant' in the original publication. Because some sites were co-dominated by ECM and AM tree species, we also included an ECM + AM category. The category comprised sites in which (1) canopy is co-dominated by ECM and AM species; or (2) canopy is dominated by a single mycorrhizal type (ECM or AM), but the understory contains a high-cover shrub layer associated with the opposite mycorrhizal type. To evaluate the potential influence of classification uncertainty, we conducted a sensitivity analysis assessing the robustness of our findings to possible misclassification among AM, ECM and ECM + AM ecosystems. At each error rate from 5% to 30%, the corresponding proportion of observations were randomly reassigned among the three ecosystem categories, and the meta-analytic model was refitted 100 times. Analyses were performed for the full dataset as well as for the short-term (<5 years) and long-term (≥5 years) N addition subsets.

To identified the important predictors of the responses of SOC to N addition in two mycorrhizal ecosystems, we also recorded environmental information, including mean annual temperature, mean annual precipitation, vegetation type (grassland vs. forest), soil types, soil pH, soil clay content, N fertilization rate, N fertilization form, N fertilization frequency and experimental duration. We distinguished short-term (<5 years) from long-term (≥5 years) N addition studies, since N effect on SOC may be time-dependent. We considered three N fertilization forms, including inorganic N ($NH_4$, $NO_3$ or $NH_4NO_3$), organic N (urea), as well as the mixture of inorganic and organic N. Moreover, to explore the underlying mechanisms of the N effect on SOC, we also collected the responses of other related variables, including soil respiration, litter inputs, soil pH, microbial biomass C, the ratio of fungi to bacteria, the ratio of soil total C to N content, and the activities of soil phosphatase, ligninase and cellulase. We also compiled the following information that was not reported in the published articles from publicly available databases: mean annual temperature and precipitation (from the WorldClim database), soil clay contents (from SoilGrids database), soil pH [from Hengl (2018)]. The unreported SD values in some articles were estimated as 1/10 of the means (Hu et al., 2024). When responses of the selected variables to N addition were only shown graphically, we digitized the data using GetData Graph Digitizer v.2.24 (http://getdata-graph-digitizer.com/).

## 2.2 | Data analysis

The response of each variable to N addition was obtained by calculating the natural log of the response ratio (ln *R*) (1):

$$\mathrm{Ln}R = \ln\left(X_N / X_C\right) = \ln\left(X_N\right) - \ln\left(X_C\right) \quad (1)$$

where $X_C$ and $X_N$ are mean of each variable in the ambient and *N* addition treatments, respectively.

The effect size of each variable was weighted by the inverse of the pooled variance ($V_i$) (2):

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

where $n_C$ and $n_N$ are the replicate numbers, $S_C$ and $S_N$ are the standard deviations of each variable in the ambient and N addition treatments, respectively.

The multilevel linear mixed-effects models were used to evaluate the effects of N addition on all variables by the 'rma.mv' function from the 'metafor' package in R (Viechtbauer, 2010):

$$\ln R_{ijk} = \beta_0 + \beta_1 \mathrm{Mycorrhizal}_{ijk} + \beta_2 \mathrm{Duration}_{ijk} + u_j + v_{jk} + e_{ijk} \quad (3)$$

where $\ln R_{ijk}$ is the effect size of observation *k* within publication *j*; $\beta_0$ is the overall intercept; $\beta_1$ and $\beta_2$ are the coefficients for mycorrhizal type and experimental duration (fixed effects); $u_j \sim N\left(0, \sigma^2_{\mathrm{Publication}}\right)$ and $v_{jk} \sim N\left(0, \sigma^2_{\mathrm{Observation}}\right)$ are the random intercepts for publication and for observation nested within publication; and $e_{ijk} \sim N\left(0, v_{ijk}\right)$ is the sampling error with known variance $v_{ijk}$.

The effects of N addition on all variables are reported as percentage change to ease interpretation, while these effects were considered significant if the 95% confidence interval of the response values did not overlap with zero. We constructed linear contrasts of the model coefficients to compare the response of SOC to N addition (Ln *R*-SOC) between ECM and AM ecosystems under short-term (<5 years) and long-term (≥5 years) N addition. These contrasts were tested using the 'anova' function in the 'metafor' package, and the significance of between-group differences was assessed using the QM statistic. We also identified the most important predictors of Ln *R*-SOC in AM and ECM ecosystems according to a meta-analytic model through the R package 'glmulti' (Calcagno & de Mazancourt, 2010). Statistical significance of each predictor was assessed using the *p* value associated with its model-averaged coefficient. A two-tailed z test was performed by dividing the model-averaged coefficient by its unconditional standard error, with $p < 0.05$ considered significant (Burnham & Anderson, 2002; Lukacs et al., 2010). To further examine the relationships between Ln *R*-SOC and important predictors, we fitted linear mixed-effects meta-regression models separately for ECM and AM ecosystems. In each model, the predictor of interest was included as a fixed effect, and observations were weighted by the inverse of their sampling variances Vi of each Ln *R*-SOC. To account for the non-independence of multiple observations within the same study, observation identity was nested within publication as random effects. Models were fitted using the 'rma.mv' function from the 'metafor' package in R (Viechtbauer, 2010).

# 3 | RESULTS

Based on the corrected Akaike Information Criterion, we quantified experimental duration as the best predictor of the effect of N addition on SOC content across all studies (Figure S2a). However, using the mixed linear regression model, we showed a significant

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interactive effect of N addition duration and mycorrhizal type on SOC content (Tables S1 and S2). Indeed, in short-term (<5 years) and long-term (≥5 years) N addition studies, we found that responses of SOC content to N addition were best predicted by mycorrhizal type (Figure 1a,b). While the response of SOC content to N addition in AM ecosystems was best predicted by experimental duration (Figure 1c), we found that in ECM ecosystems it was best predicted by experimental duration and mean annual temperature (Figure 1d).

Specifically, in AM ecosystems, there was a lack of significant changes of SOC content and even a decreasing tendency for SOC stock in short-term N addition studies (Figure 2a,c). In long-term N addition studies, SOC content and stock in AM ecosystems significantly increased by 7.3% (95% CIs, 5.8% to 8.7%, $p < 0.001$) and by 12.7% (7.1% to 18.6%, $p < 0.001$), respectively. In ECM ecosystems, N addition significantly increased SOC content by 4.1% (2.5% to 5.7%, $p < 0.001$) and SOC stock by 9.1% (6.5% to 11.9%, $p < 0.001$) regardless of whether the studies were short- and long-term. Additionally, ecosystems co-dominated by ECM and AM plants exhibited response patterns similar to those observed in AM ecosystems, with no significant changes in SOC under short-term N addition (Figure S3).

Sensitivity analysis further confirmed the robustness of our findings to potential misclassification of mycorrhizal ecosystems. Specifically, results from the full dataset were highly robust, with pooled effect sizes for all three mycorrhizal categories remaining within ±13% of original estimates even at a 30% misclassification rate (Figure S4a). Similar robustness was observed for the long-term N addition subsets, where the dominant SOC responses of the AM and ECM+AM categories were maintained, with deviations <12% at the highest simulated error rate of 30% (Figure S4b). In contrast, the short-term subset was somewhat more sensitive to misclassification, with effect-size estimates for AM and ECM ecosystems gradually converging toward a common mean as the error rate increased (Figure S4c). Nevertheless, the overall differences between AM and ECM ecosystems remained qualitatively unchanged. Furthermore, the contrasting SOC responses between AM and ECM ecosystems were also evident even when the analysis was restricted to forest ecosystems only (Figure S5).

Nitrogen addition did not change plant litter inputs but significantly reduced soil respiration in ECM ecosystems in both short- and long-term studies (Figure 3a,b). In AM ecosystems, N addition significantly increased plant litter inputs, particularly under the long-term studies (Figure 3a). Soil respiration in AM ecosystems did not change in the short-term N addition studies, but significantly reduced in the long-term N addition studies (Figure 3b). Nitrogen addition significantly decreased soil pH in both ecosystems, with a larger decline in AM ecosystems (−0.35 units) than in ECM ecosystems (−0.19 units). This contrast response between AM and ECM ecosystems was particularly pronounced under long-term N addition, where soil pH decreased by 0.52 units in AM ecosystems compared with only 0.2 units in ECM ecosystems (Figure 3c). Nitrogen addition significantly decreased

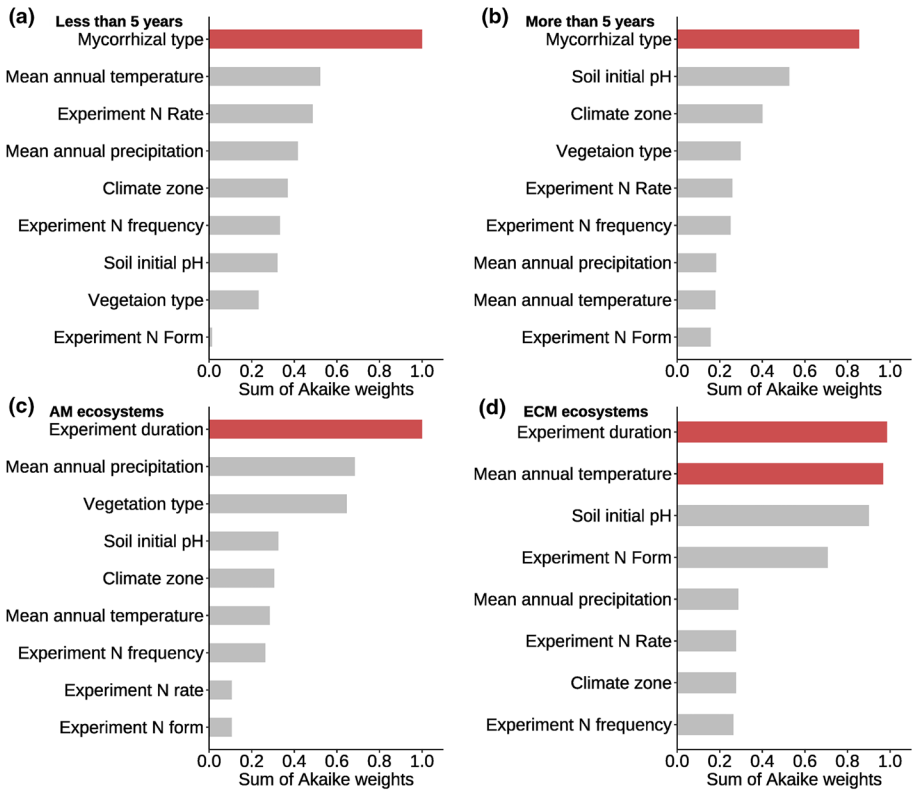



**FIGURE 1** Relative importance of predictors explaining soil organic carbon responses to nitrogen (N) addition when grouped by experimental duration (a, b) and mycorrhizal association (c, d). Bar heights represent the relative importance (sum of Akaike weights) of each predictor. Red bars denote significant predictors ($p < 0.05$), whereas grey bars indicate non-significant predictors ($p > 0.05$). ECM and AM refer to ecosystems dominated by ectomycorrhizal or arbuscular mycorrhizal plants.

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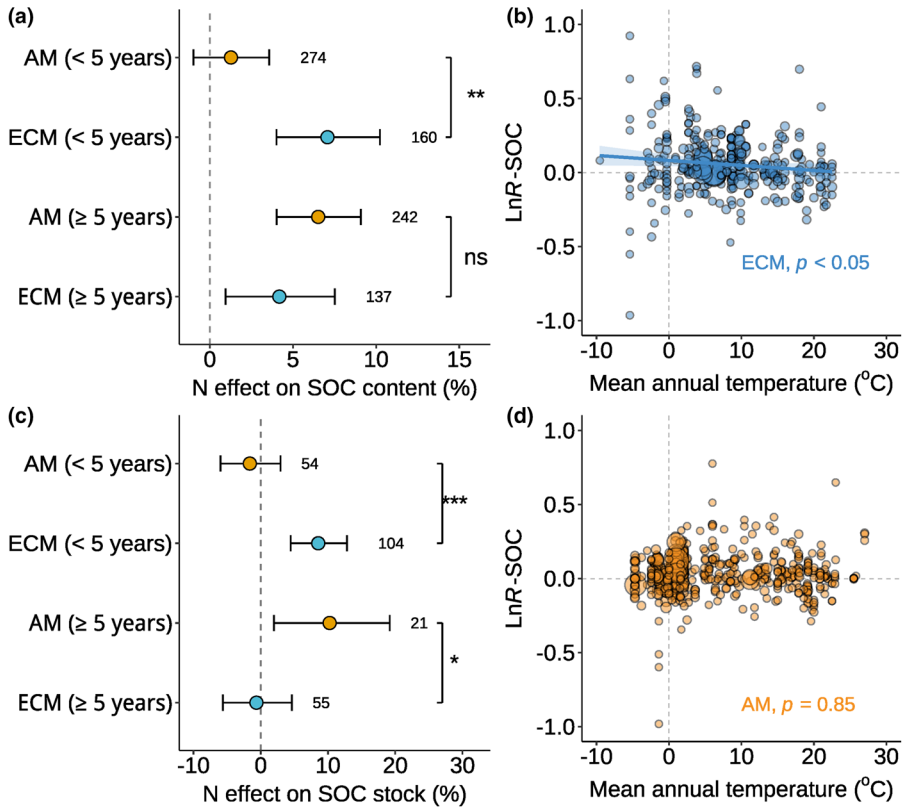



**FIGURE 2** The responses of soil organic carbon (SOC) to nitrogen (N) addition as influenced by mycorrhizal type, experimental duration and mean annual temperature. (a, c) Effects of N addition on SOC in ecosystems dominated by ectomycorrhizal (ECM) and arbuscular mycorrhizal (AM) plants under different experimental durations. Error bars represent bootstrap 95% confidence intervals; numbers near the error bars are sample sizes; asterisks denote significant differences in SOC to N addition (Ln *R*-SOC) between ECM and AM ecosystems, assessed using the QM statistic (ns, $p > 0.05$; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$). (b, d) Relationships between Ln *R*-SOC to mean annual temperature in AM and ECM ecosystems. Relationships were assessed using a linear mixed-effects model, with observation nested within publication as random effects included as random effects and each effect size weighted by the sampling variance (vi) of SOC. Shaded areas indicate 95% confidence intervals around the fitted regression lines. ECM and AM denote ecosystems dominated by ectomycorrhizal or arbuscular mycorrhizal plants, respectively.

microbial biomass C only in AM ecosystems (Figure S6). However, N addition significantly decreased the fungi to bacterial ratio only in ECM ecosystems (Figure S6). Additionally, N addition significantly decreased soil ligninase activity but increased soil cellulase activity only in ECM ecosystems (Figure 4). Moreover, the decreased ligninase activity under N addition was negatively correlated with the responses of SOC in ECM ecosystems (Figure 4). Nitrogen addition significantly increased soil phosphatase activity in the short-term studies only in AM ecosystems, but over the long-term studies, becoming no significant change (Figure 4). The response of soil phosphatase activity was negatively related with the responses of SOC in AM ecosystems.

## 4 | DISCUSSION

### 4.1 | Nitrogen addition effects on SOC in AM ecosystems

We found a significant increase in SOC across all N addition studies conducted in AM ecosystems, which contradicts the results of a previous meta-analysis that suggested a decreasing tendency for SOC in these ecosystems (Yang et al., 2023). This discrepancy probably arises from our study including data based on a more extensive N addition duration than their study (only one study more than 5 years in a total of 21 studies in AM forests). In AM ecosystems, the significant increase in SOC can indeed only be found in the long-term N addition, while there is a lack of significant changes in SOC content and even a decreasing tendency for SOC stock in short-term N addition studies (Figure 2). Although short-term N addition decreased soil pH by 0.19 units ($p < 0.05$) in AM ecosystems, a change that is often associated with suppressed microbial decomposition, it may simultaneously stimulate microbial activity by alleviating N limitation (Treseder, 2008). Besides, N-induced faster plant growth in AM ecosystems could increase plant P demand, leading to a priming effect that accelerates microbial decomposition. Indeed, we found a significant increase in soil phosphatase activity under short-term N addition in AM ecosystems, which was negatively related to the response in SOC. Moreover, in the early stages of N addition, increased C input in AM ecosystems is often related to less efficient decomposition and lower C stabilization (Pregitzer et al., 2004; Xu et al., 2021). Thus, although we found a slight increase in plant litter inputs in

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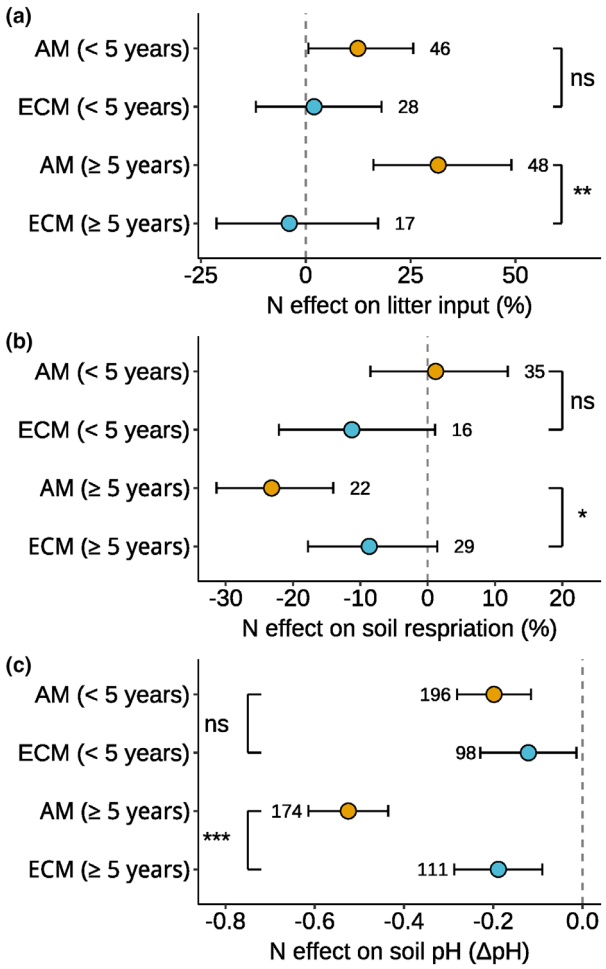



**FIGURE 3** Effects of nitrogen addition on plant litter inputs (a), soil respiration (b) and soil pH (c) when grouped by mycorrhizal type and experimental duration. ECM and AM denote ecosystems dominated by ectomycorrhizal and arbuscular mycorrhizal plants. Error bars represent bootstrap 95% confidence intervals; the numbers near the error bars are sample sizes. Asterisks denote significant differences in the response variables between ECM and AM ecosystems, assessed using the QM statistic (ns, $p > 0.05$; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

AM ecosystems, it may not result in an increased SOC under short-term N addition.

In long-term (≥5 years) N addition studies, the increases in SOC content and stock in AM ecosystems were significantly greater than those of ECM ecosystems (Figure 2). This N-increased SOC accumulation in AM ecosystems could be partly attributed to cumulative increase in litter inputs in the later stage of N addition (Figure 3a). A comprehensive meta-analysis suggested that significant increases in both above-ground and below-ground litter inputs under N addition may last for more than decades (Xu et al., 2021). On the other hand, in the later stage of N addition, AM plants and microorganisms may gradually adapt to N-induced P limitation, resulting in the diminished positive effects on microbial decomposition and C losses from their increased P demand (Mei et al., 2019). Moreover, in AM ecosystems, N addition may inhibit microbial activity and C losses in the later stage due to progressive soil acidification and osmotic stress (Zhou et al., 2014) (Figure 3c). Under long-term N addition, soil respiration significantly decreased in AM ecosystems but not in ECM ecosystems, which may partly explain the greater SOC accumulation as observed in AM ecosystems (Figure 3b). This acidification-induced depression in soil respiration may further explain why long-term N addition enhanced SOC accumulation in N-rich (tropical) AM ecosystems while only having minor effects on plant growth (Lu et al., 2021; Figure S7). We acknowledged that heterotrophic respiration provides a more direct measure of microbially mediated C loss. However, because data on heterotrophic respiration were too limited to support a robust analysis, we used total soil respiration as a proxy instead (Figure S8). Overall, the decrease in N-induced P limitation accompanied by increases in litter inputs and soil acidification over time together contributed to the increased SOC in AM ecosystems under long-term N addition.

## 4.2 | Nitrogen addition effects on SOC in ECM ecosystems

In agreement with our first hypothesis, N addition significantly increased SOC in ECM ecosystems under both short- and long-term conditions through mechanisms markedly different from those in AM ecosystems (Figure 5). Unlike AM ecosystems, N addition had no significant effect on plant litter inputs but significantly reduced soil respiration in ECM ecosystems, suggesting that the increase in SOC was primarily driven by suppressed microbial decomposition (Figure 3). Furthermore, in contrast to AM ecosystems, the reduction in microbial decomposition in ECM ecosystems may not be solely attributed to N-induced soil acidification. Soil acidification typically changes microbial community composition, often resulting in a higher ratio of fungi to bacteria, as fungi tend to have a relatively lower pH optimum than bacteria (Högberg et al., 2007). However, across all N addition studies in ECM ecosystems, soil pH decreased only modestly, from 5.49 to 5.30, remaining within the acidic range generally favourable for fungal growth. Consistent with this, N addition significantly reduced the ratio of fungi to bacteria in ECM ecosystems (Figure S6). This response may reflect the ecological characteristics of ECM tree species, which are primarily distributed in boreal and temperate regions where soils are usually alkaline to circumneutral pH. In these soils, acidification is mostly buffered by replacement of base cations on ion exchange sites (Kirk et al., 2010), even possibly releasing C bound to clay minerals via divalent cations.

Alternatively, unlike AM fungi, ECM fungi can produce lignin-degrading enzymes such as ligninase that catalyse the degradation of complex organic matter. The production of these enzymes is often suppressed when N limitation is alleviated by N addition, thereby reducing soil C loss (Carrara et al., 2021; Pellitier & Zak, 2021). Consistent with this mechanism, we found a significant decline in ligninase activity only in ECM ecosystems, but not in AM ecosystems, and SOC responses were negatively correlated with ligninase activity under N addition (Figure 4). The preferential accumulation of stable SOC in ECM rather than AM ecosystems under N addition is also supported

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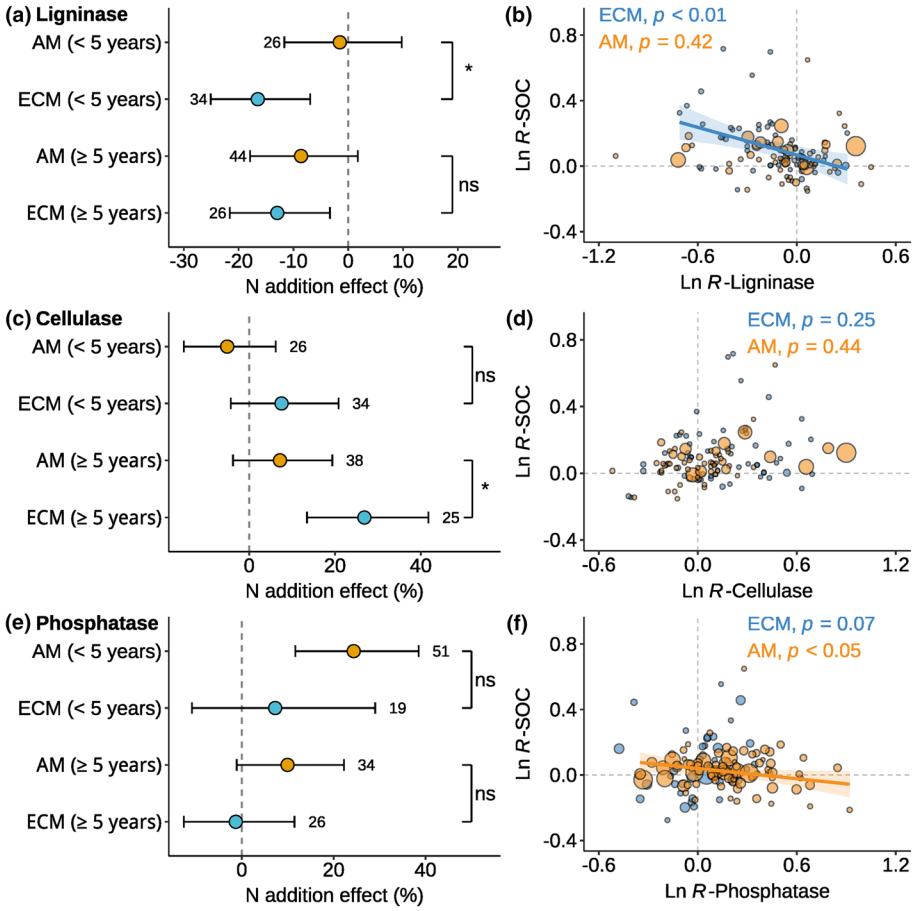



FIGURE 4 Effects of nitrogen addition on the activities of soil ligninase, cellulase and phosphatase when grouped by mycorrhizal type and experimental duration and their relationships with soil organic carbon responses. (a, c, e) Responses of ligninase, cellulase and phosphatase activities to N addition in ecosystems dominated by ectomycorrhizal (ECM) and arbuscular mycorrhizal (AM) plants under different experimental durations. Error bars represent bootstrap 95% confidence intervals, and numbers adjacent to the error bars are sample sizes. Asterisks indicate significant differences in responses of soil organic carbon (Ln *R*-SOC) between ECM and AM ecosystems, assessed using the QM statistic (ns, $p > 0.05$). (b, d, f) Relationship between Ln *R*-SOC to mean annual temperature in AM and ECM ecosystems was assessed using a linear mixed-effects model, with observation nested within publication as random effects included as random effects and each effect size weighted by the sampling variance (vi) of SOC. Shaded areas represent 95% confidence intervals around the fitted regression lines. ECM and AM denote ecosystems dominated by ectomycorrhizal or arbuscular mycorrhizal plvants.

by a previous meta-analysis (Hu et al., 2024). However, N addition may also weaken competition for N between ECM fungi and soil free-living decomposers, potentially stimulating the microbial degradation of less stable SOC fractions (Averill et al., 2014). Indeed, we found no significant change in microbial biomass C but a significant increase in cellulase activity under long-term N addition in ECM ecosystems (Figure 4; Figure S6). In contrast, AM ecosystems exhibited reduced microbial biomass C and soil respiration under long-term N addition. These results suggest that enhanced decomposition of relatively labile SOC fractions may partially offset C gains in ECM ecosystems, which could explain why the long-term increase in SOC was slightly lower in ECM than in AM ecosystems (Figure 2).

The response of SOC to N addition in ECM ecosystems was best predicted by MAT, with the N-induced SOC negatively correlated with MAT (Figure 1d). Compared to warmer regions, soils in colder regions are often characterized by slower N cycling and lower levels of inorganic N, probably resulting in greater N limitation for plants and stronger interactions between plants and microbes for N acquisition (Du et al., 2020). Thus, the importance of MAT in predicting the effects of N addition on SOC likely stems from the fact that N-alleviated N limitation caused a greater reduction in plant–microbe interactions for N acquisition in colder regions, whereas these effects may be less pronounced in warmer regions. Indeed, we did not find significant effects of N addition on SOC in tropical ECM ecosystems (Figure S7). Additionally, we found that the response of ligninase activity to N addition in ECM ecosystems was negatively related to MAT, which may further explain why the positive response of SOC to N addition decreased with MAT (Figure 2b).

## 4.3 | Implication

Our results demonstrate that, overall, N addition enhanced SOC in both AM and ECM ecosystems, yet the underlying mechanisms

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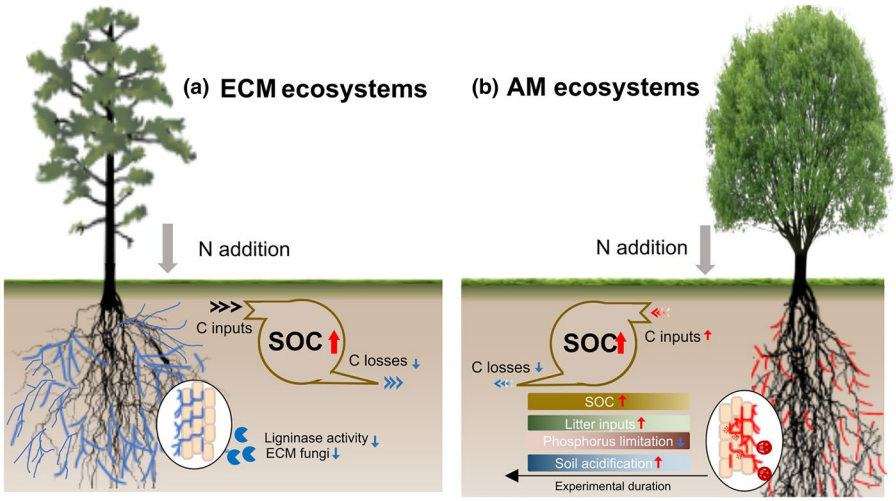



**FIGURE 5** Conceptual framework illustrating the mechanisms underlying nitrogen (N)-induced increases in soil organic carbon (SOC) content in ecosystems dominated by different mycorrhizal associations. (a) In ectomycorrhizal (ECM) ecosystems, N addition enhances SOC primarily through the suppression of ligninase activity. (b) In arbuscular mycorrhizal (AM) ecosystems, SOC accumulation under N addition is driven by progressively increasing litter inputs, increasing soil acidification and gradual alleviation of N-induced phosphorus (P) limitation. ECM and AM denote ecosystems dominated by ectomycorrhizal and arbuscular mycorrhizal plants, respectively; ≥5 years and <5 years indicate N addition experimental duration of more than 5 years and less than 5 years, respectively.

in the two ecosystems may be different (Figure 5). Specifically, the enhanced SOC in AM ecosystems was primarily attributed to progressive increases in litter inputs and soil acidification as well as the progressive declines in N-induced P limitation. However, in ECM ecosystems where N addition did not change litter inputs, SOC also enhanced significantly due to the reduced ECM fungi for N acquisition, which resulted in a shift of soil microbial communities and reduction in ligninase activity. More importantly, our results further identified the distinct temporal N effect on SOC in AM and ECM ecosystems, possibly implying that the potentials of soil C sequestration differ between two ecosystems from short- to long-term N addition (Figure 2). Under N addition, soils in ECM ecosystems may accumulate C quite quickly, but the accumulation rate over long-term (> years) would be lower than AM ecosystems. Previous studies did not identify mycorrhizal associations as an important driver (Zhang et al., 2025), likely due to overlooked interactions with N addition duration (Tables S1 and S2). Moreover, it is crucial to recognize that our study is restricted to ecosystems dominated by a single mycorrhizal type. Predicting future terrestrial C sinks will therefore require understanding how SOC responds to N deposition along continuous gradients of mycorrhizal dominance, especially as global change shifts the distribution of mycorrhizal associations.

## AUTHOR CONTRIBUTIONS

Qi Deng and Ji Chen conceived the ideas; Yuanliu Hu, Qi Deng and Ji Chen designed the methodology; Yuanliu Hu, Qi Deng, Ji Chen, Guoyin Chen, Yiren Zhu, Xiaolin Huang and Jianling Li collected the data; Yuanliu Hu, Qi Deng and Ji Chen analysed and visualized the data; Qi Deng and Ji Chen supervised the project; Yuanliu Hu led the writing of the manuscript, and Qi Deng, Ji Chen, Dafeng Hui, Richard P. Phillips, Ying-Ping Wang and Yiqi Luo reviewed and edited the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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## CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests. Ji Chen is a Senior Editor of Functional Ecology. He took no part in the peer review and decision-making processes for this paper.

## DATA AVAILABILITY STATEMENT

Data available from the figshare Digital Repository: https://doi.org/10.6084/m9.figshare.32719776 (Hu et al., 2026).

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## SUPPORTING INFORMATION

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

**Text S1.** PRISMA-flowchart of assessment of eligible studies.

**Table S1**. Mixed-effect model was used to identify predictors that significantly affected the responses of soil organic carbon (SOC) to nitrogen (N) addition. Because model selection analyses identified experiment duration as the most important predictor of the responses of SOC to N addition, the interaction effects between the experiment duration and other predictors are considered in this mixed-effect model.

**Table S2**. Mixed-effect models were used to identify the importance of the interaction between experiment duration and other predictors in the responses of soil organic carbon to nitrogen (N) addition.

**Figure S1**. Distribution of nitrogen addition experiments in this meta-analysis. (a) Global distribution of nitrogen addition experiments in this meta-analysis. (b–d) Density distribution of the response of soil organic carbon (SOC) to nitrogen addition when grouped by mycorrhizal association, climate zone and vegetation type, respectively. Ln *R* is the response ratio; ECM and AM indicate ecosystems dominated by ectomycorrhizal and arbuscular mycorrhizal plants, respectively; ECM + AM, ecosystems co-dominated by ectomycorrhizal and arbuscular mycorrhizal plants.

**Figure S2**. Predictors of soil organic carbon responses to nitrogen (N) addition across all studies. Bars show the relative importance (sum of Akaike weights) of each predictor. Red bars indicate significant predictors ($p < 0.05$), whereas grey bars indicate non-significant predictors ($p > 0.05$).

**Figure S3**. Effects of nitrogen (N) addition on soil organic carbon (SOC) between ecosystems dominated by ectomycorrhizal (ECM), arbuscular mycorrhizal (AM) and mixture of both ECM + AM plants. Error bars represent bootstrap 95% confidence intervals; numbers near the error bars are sample sizes.

**Figure S4**. Sensitivity of pooled effect sizes of soil organic carbon (SOC) to simulated misclassification of mycorrhizal type at the full dataset (a), short-term (b) and long-term (c) nitrogen addition subsets. Dashed lines indicate the original pooled effect size estimated without misclassification. RR is response ratio; ECM and AM indicate ecosystems dominated by ectomycorrhizal and arbuscular mycorrhizal plants, respectively; ECM + AM denote ecosystems co-dominated by ectomycorrhizal and arbuscular mycorrhizal plants.

**Figure S5**. The important predictors in the responses of soil organic carbon to nitrogen addition in forest ecosystems when grouped by mycorrhizal type. (a) Bars show the relative importance (sum of Akaike weights) of each predictor. Red bars indicate significant predictors ($p < 0.05$), whereas grey bars indicate non-significant predictors ($p > 0.05$). (c) Error bars represent bootstrap 95% confidence intervals; numbers near the error bars are sample sizes; asterisks indicate the significant difference in SOC to N addition (Ln R-SOC) between ECM and AM ecosystems, assessed using the QM statistic (ns, $p > 0.05$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). ECM and AM indicate ecosystems dominated by ectomycorrhizal and arbuscular mycorrhizal plants, respectively.

**Figure S6**. Effects of nitrogen addition on the ratio of fungal-to-bacterial biomass (F:B), soil microbial biomass carbon content (MBC) and the ratio of soil C-N ratio (TC:TN) when grouped by mycorrhizal type. ECM and AM indicate ecosystems dominated by ectomycorrhizal and arbuscular mycorrhizal plants, respectively; error bars represent bootstrap 95% confidence intervals; the numbers near the error bars are sample sizes.

**Figure S7**. Effects of nitrogen addition on soil organic carbon (SOC) between ecosystems dominated by ectomycorrhizal and arbuscular mycorrhizal plants when grouped by and climate zone. ECM and AM indicate ecosystems dominated by ectomycorrhizal and arbuscular mycorrhizal plants, respectively; error bars represent bootstrap 95% confidence intervals; the numbers above or below the error bars are sample sizes.

**Figure S8**. Effects of nitrogen addition on soil heterotrophic respiration when grouped by mycorrhizal type and experimental duration. ECM and AM indicate ecosystems dominated by ectomycorrhizal and arbuscular mycorrhizal plants, respectively; error bars represent bootstrap 95% confidence intervals; the numbers near the error bars are sample sizes.

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