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Chen, X., Cao, J., Sinsabaugh, R.L. et al. (6 more authors) (2025) Soil extracellular enzymes as drivers of soil carbon storage under nitrogen addition. Biological Reviews. ISSN 1464-7931

https://doi.org/10.1111/brv.70021

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Soil extracellular enzymes as drivers of soil carbon storage under nitrogen addition

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ABSTRACT

Enhanced anthropogenic nitrogen (N) inputs to ecosystems may have substantial impacts on microbially mediated soil organic carbon (SOC) cycling. One way to link species-rich soil microbial communities with SOC cycling processes is *via* soil extracellular enzyme activities (EEAs). However, the effects of N addition on EEAs and the associated driving factors remain poorly understood. By conducting a meta-data synthesis, we find that N addition increases hydrolytic C-degrading EEAs that target simple polysaccharides by 12.8%, but decreases oxidative C-degrading EEAs that degrade complex phenolic macromolecules by 11.9%. The net effect of N addition on SOC storage is determined by the shifts between these two types of C-degrading EEAs, and the impacts varied across different ecosystem types. These insights highlight the crucial but understudied roles of hydrolytic and oxidative C-degrading EEAs on SOC dynamics with ongoing enhanced anthropogenic N loading. Understanding the mechanisms behind these C-degrading EEAs could help optimize SOC sequestration and inform climate mitigation strategies across different ecosystems.

Key words: nitrogen addition, hydrolytic C-degrading enzyme activities, oxidative C-degrading enzyme activities, soil microorganisms, soil carbon storage, meta-data synthesis.

CONTENTS

I. INTRODUCTION

Reactive nitrogen (N) emissions to the atmosphere have increased substantially over the past decades in most regions, primarily due to human activities (Fenn *et al.*, 2018; Liu *et al.*, 2013). This escalation exhibits significant spatial variation among regions (Liu *et al.*, 2016; Schwede *et al.*, 2018). Increasing N deposition to the land surface has significantly increased the productivity of terrestrial ecosystems, notably due to the alleviation of N limitation (Mason *et al.*, 2022). Furthermore, N availability is key to ecosystem functioning, the cycling of nutrients and the flow of energy through the biosphere, with profound impacts on soil organic carbon (SOC) decomposition in terrestrial ecosystems (Ye *et al.*, 2018).

Despite numerous studies in recent decades, the net effects of N addition on SOC storage are controversial. Recent meta-analyses of N addition experiments at global or regional scales have shown that N addition can increase SOC storage by 4–11% (Chen *et al.*, 2018*a*; Hu *et al.*, 2024*a*,*b*; Janssens *et al.*, 2010; Liu & Greaver, 2010; Xu *et al.*, 2021; Yue *et al.*, 2017). This increase in SOC storage may occur because N inputs can alleviate N limitation for microbial decomposers and decrease microbial degradation of pre-existing SOC for N acquisition (i.e. negative priming effect and reduced N mining from organic matter) (Craine, Morrow & Fierer, 2007; Hicks, Lajtha & Rousk, 2021; Kuzyakov, 2010). Conversely, N addition could increase soil N availability and the activity of previously N-limited microbial decomposers, thereby enhancing SOC decomposition and reducing SOC storage (Zhang *et al.*, 2014). N addition may lead to soil acidification. It can indirectly impact SOC storage by inhibiting microbial growth (Ontman *et al.*, 2023), reducing enzyme efficiency (Chen *et al.*, 2017), decreasing necromass accumulation (Ye *et al.*, 2018), and affecting soil structure (Cotrufo *et al.*, 2015; Liang, Schimel & Jastrow, 2017). These combined effects can lead to a long-term decrease in SOC storage. Several mechanisms may help explain changes in SOC storage following N addition, including altered C inputs due to a change in net primary productivity (Čapek *et al.*, 2018), changes in microbial activity (Crowther *et al.*, 2019) and/or changes in soil aggregation (Lu *et al.*, 2021*a*), but a comprehensive understanding of the overall effects of N addition on SOC decomposition remains elusive. Specifically, the lack of a mechanistic understanding hinders our ability to predict long-term effects of N addition on SOC dynamics.

Changes in SOC storage resulting from N addition are associated with a range of soil microbial properties, including community composition, physiology, and metabolic activities (Jing *et al.*, 2021; Ni *et al.*, 2021). However, establishing a direct link between diverse soil microbial properties and changes in SOC storage remains a formidable challenge due to the intricate feedbacks between plants, soil, and microbes (Chen, Sinsabaugh & van Groenigen, 2023). Extracellular enzymes are produced by plants and soil microorganisms, providing another perspective for understanding the effects of N addition on SOC storage. Soil extracellular enzyme activities (EEAs) mediate the transformation of SOC by catalysing rate-limiting steps in SOC decomposition (Fanin *et al.*, 2022; Sinsabaugh, 2010), which may directly or indirectly affect SOC storage. Hydrolytic and oxidative C-degrading EEAs are among the most important C-degrading enzymes targeting the degradation of litter and SOC with different qualities. Hydrolytic C-degrading enzymes catalyse the degradation of cellulose, whereas oxidative C-degrading enzymes facilitate the biodegradation of lignin and other phenolic compounds in litter and soil (Ljungdahl & Eriksson, 1985). Hydrolytic and oxidative C-degrading EEAs can provide another mechanistic insight to track SOC dynamics (Chen *et al.*, 2017) and the ratio of oxidative to hydrolytic C-degrading EEAs provides an index that reflects microbial preference in utilizing various pools of litter and SOC (Yang *et al.*, 2019). It is important to note that geography significantly influences the soil's physical and chemical properties (Islam *et al.*, 2020; Lira-Martins *et al.*, 2022). These soil characteristics, in turn, can impact the abundance, diversity, and activity of soil microbes (Choe, Kim & Lee, 2021; Islam *et al.*, 2020; Carson *et al.*, 2007). For instance, soils rich in specific minerals may foster the growth of certain microbial communities, thereby influencing the overall pattern of EEAs (Choe *et al.*, 2021; Carson *et al.*, 2007). Therefore, understanding the responses of soil C-degrading EEAs to N addition is central to predicting changes in SOC cycling and its feedback to climate change, which may offer fresh insights to advance the understanding of the intricate plant–soil–microbial feedback on SOC storage under N addition.

In this study, we performed a comprehensive global meta-data synthesis of the responses of soil C-degrading EEAs to N addition. This study aims to provide novel insights into the role of soil EEAs in mediating the response of SOC storage to N addition. Several previous meta-analyses have synthesized the impact of N addition on soil C-acquisition EEAs (Chen *et al.*, 2017; Jia *et al.*, 2020; Jian *et al.*, 2016; Xiao *et al.*, 2018), but the links between soil EEAs and SOC storage have not been explicitly examined, let alone the underlying mechanisms. Additionally, there has been a surge in field studies on soil C-acquisition EEAs related to N addition over recent years, providing a unique opportunity to examine the links between soil EEAs and SOC storage. To this end, we conducted a comprehensive synthesis of data from multiple field experiments that concurrently measured C-degrading EEAs and SOC in response to N addition (Allison *et al.*, 2010; Du *et al.*, 2014; Luo *et al.*, 2019; Rappe-George *et al.*, 2017; Zhu *et al.*, 2020). A meta-data synthesis approach was combined with a state-of-the-art model selection analysis to evaluate simultaneously the importance of a range of biotic and abiotic factors on the effects of N addition on SOC and C-degrading EEAs. In particular, we explored the role of C-degrading EEAs related to changes in SOC storage by analysing data from N addition studies that included both SOC storage and C-degrading EEAs. Based on previous experimental and synthesis studies, we hypothesized that: (1) N addition suppresses C-degrading EEAs due to altered N availability; and (2) N addition increases SOC storage, which is associated with N-induced changes in C-degrading EEAs.

II. METHODS

(1) Data collection

We systematically searched all peer-reviewed journal articles that investigated the effects of N addition on SOC storage as well as the underlying enzymatic mechanisms, using *Web of Science* (http://apps.webofknowledge.com/), *Google Scholar* (http://scholar.google.com/), and *China National Knowledge Infrastructure* (CNKI, www.cnki.net/) up to October 1st, 2022. The literature search was performed following guidelines from Preferred Reporting

Items for Systematic Reviews and Meta-Analyses (see online Supporting Information, Appendix S1 for PRISMA flow chart). Various key word combinations were used for the search: (nitrogen addition OR nitrogen amendment OR nitrogen enrichment OR nitrogen fertilizer OR nitrogen elevated OR nitrogen deposition) AND (glucosidase OR cellobiosidase OR xylosidase OR peroxidase OR phenol oxidase OR polyphenol oxidase OR lignin modifying enzymes OR cellulase) AND (soil carbon) AND (terrestrial OR soil OR land). Additional studies that were not covered by these search terms but were cited in Chen *et al.* (2018*b*) were also included. We reviewed each article to determine whether the studies met the following criteria: (1) articles must have an N addition treatment with a paired ambient treatment (control). For multifactorial studies, only ambient and N addition treatments (including multiple N addition levels) were included. Data from N addition treatments combined with other factors (e.g. P addition) were excluded. (2) Ambient and N addition treatments must be conducted at the same experimental site, thus the microclimate, ecosystem type, and soil types are similar between ambient and N addition treatments. (3) Details of N addition methods (rate, frequency, form, and duration) must be provided. (4) Activity of at least one kind of C-degrading EEA must be measured. (5) The experiment must be conducted in the field. Studies involving model simulations and incubations in the laboratory (e.g. greenhouse or pot experiments) were not included. We used Plot Digitizer version 2.0 to digitally extract data from figures when the results were graphically reported. When data from different years were available in the same study, we used the latest data in accordance with the commonly used approach in meta-data synthesis studies (Lu *et al.*, 2021*a*,*b*). When data for the same variable from multiple soil layers were provided, we only used data from the uppermost soil horizon to maintain independence across the different studies.

Following the criteria mentioned above, we obtained a meta-data set of 379 paired observations from 102 publications (see Appendix S2 for full list of included studies). In brief, the variables examined in this study included C-degrading EEAs (listed in Table 1), plant properties (expressed as plant productivity, e.g. aboveground biomass, diameter at breast height, root biomass, litter biomass, net primary productivity), soil properties (soil pH, SOC, recalcitrant SOC, soil total N and the ratio of SOC to N) and microbial properties [microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), MBC:MBN, bacterial and fungal biomass, and the ratio of fungal to bacterial biomass]. The following indicators of SOC recalcitrance were used: (*i*) soil lignin content or the size of the slow-decomposing C pool; and (*ii*) the heavy fraction of C or non-hydrolyzable organo-mineral complexed C. To estimate the effect of N addition on microbial biomass, we prioritized the following proxies based on their availability: (1) for MBC and MBN, we tabulated microbial biomass measured by chloroform fumigation (Brookes *et al.*, 1985; Vance, Brookes & Jenkinson, 1987); (2) for bacterial biomass, we tabulated microscopic counts in soil extracts, bacterial phospholipid fatty acid (PLFA) concentrations (Bossio & Scow, 1998; Frostegård, Tunlid & Bååth, 2011) or quantitative PCR analysis; and (3) for fungal biomass, we used microscopic counts in soil extracts, fungal PLFA concentrations, or soil concentrations of ergosterol. Results for specific groups of fungi or bacteria (e.g. arbuscular mycorrhizal fungi, saprotrophic fungi, ammonia-oxidizing bacteria/archaea, and actinomycetes) were excluded to avoid biasing results towards subsets of the fungal or bacterial communities. In our analysis, hydrolytic C-degrading EEAs were represented by the activities of β-1,4-glucosidase, β-1,4-xylosidase, and β-1,4-D-cellobiohydrolase, or a combination of these. These hydrolytic C-degrading enzymes accelerate the breakdown of cellulose or hemicellulose. Oxidative C-degrading EEAs were represented by the activities of peroxidase, phenol oxidase, polyphenol oxidase, or a combination of these (Table 1). These oxidative C-degrading enzymes mediate the breakdown of relatively recalcitrant molecules such as lignin, phenols, and other aromatics. The above enzymes are among the most important for the degradation of litter and SOC (Chen *et al.*, 2017; Jian *et al.*, 2016; Margida, Lashermes & Moorhead, 2020). In instances where researchers reported the presence of multiple types of hydrolytic and oxidative C-degrading EEAs, the sum of their values was used to represent the cumulative response of hydrolytic or oxidative C-degrading EEAs (Chen *et al.*, 2018*b*).

In our data set, we also recorded a wide range of environmental variables, including latitude (30.4°S to 69.0°N), longitude (145.7°W to 137.9°E), mean annual temperature (MAT, –5.4 to 24.8 °C), mean annual precipitation (MAP, 69–3537 mm), initial soil pH (3.2–8.7), initial SOC (3.3–179.1 $\mathrm{g \cdot kg^{-1}}$) and initial total nitrogen (TN, 0.43–12.7 $\mathrm{g \cdot kg^{-1}}$). The global distribution of the experimental sites is presented in Fig. 1. If these data were not reported, we contacted the corresponding author for more information. Otherwise, we obtained MAT and MAP from the *WorldClim* database (www.worldclim.org/), and background N deposition from the *Global N deposition* database (http://webmap.ornl.gov/). We classified ecosystem types according to the Whittaker Biome Diagram (Whittaker, 1962), and soil types according to the Food and Agriculture Organization taxonomy (www.fao.org/soils-portal/soil-survey/soil-classification/usda-soil-taxonomy/en).

(2) Data analysis

We quantified the effects of N addition on the studied variables using response ratios (RRs), which were calculated for each observation by taking the natural logarithm of the average EEAs of the ambient and N addition treatments as described in Equation (1), in which $\overline{X_\mathrm{t}}$ and $\overline{X_\mathrm{c}}$ are the respective means of a given variable in the N addition treatment group and the ambient (control) group, respectively.

$$\mathrm{RR} = \mathrm{In}\frac{\bar{x}_\mathrm{t}}{\bar{x}_\mathrm{c}} \qquad (1)$$

The variance (v) of RR was calculated following Equation (2), where $\mathrm{SD_t}$ and n_t are the standard deviation and sample size of a given variable observed in the treatment group, and $\bar{\mathrm{S}}\mathrm{D}_{\mathrm{c}}$ and n_{c} are the respective mean, standard deviation and sample size of a given variable in the control group.

$$v = \frac{\mathrm{SD}_{\mathrm{c}}^{2}}{n_{\mathrm{c}} \times \bar{x}_{\mathrm{c}}^{2}} + \frac{\mathrm{SD}_{\mathrm{t}}^{2}}{n_{\mathrm{t}} \times \bar{x}_{\mathrm{t}}^{2}} \qquad (2)$$

The effects of N addition on plant productivity, soil characteristics, C-degrading EEAs and microorganisms were evaluated by mixed-effects models using the *rma.mv()* function from the R package *metafor* (Viechtbauer, 2010). The random factor ‘experiment’ was included to ensure independence among multiple RRs within an experiment (an experiment may have included several N addition rates). Previous meta-analyses suggest that the response of C-related processes to N enrichment can vary with ecosystem types and fertilization regimes (Chen *et al.*, 2015; Chen *et al.*, 2018*b*; Deng *et al.*, 2018). Thus, all observations were subdivided into four categories based on ecosystem type (forest, grassland, farmland and shrubland) and various N addition properties, including N addition rate (high, >10 g N m^{-2} $year^{-1}$; medium, 5–10 g N m^{-2} $year^{-1}$; low, <5 g N m^{-2} $year^{-1}$), experimental duration (<5 years, 5–10 years, and >10 years), N addition frequency (< 4 times $year^{-1}$, 4–12 times $year^{-1}$ and > 12 times $year^{-1}$), and N fertilizer form [mix; organic, i.e. urea; inorganic, i.e. NH_4NO_3, $NaNO_3$, NH_4Cl, $(NH_4)_2SO_4$]. We chose these thresholds for breakpoints based on results from previous studies (Chen *et al.*, 2018*b*; Lu *et al.*, 2021*a*; Song *et al.*, 2019) and general breakpoints used in manipulative N-addition experiments in our data set. The statistical results reported include total heterogeneity among all observations, the heterogeneity in the RRs associated with each moderator variable (Q_M), and the residual error. A significant Q_M indicates a significant effect of the moderator variable on the RR (Hedges, Gurevitch & Curtis, 1999). The effects of N addition were considered significant if the 95% confidence interval did not overlap with zero. The results are reported as percentage change with N addition [i.e.100% × (e^{RR}–1)] to aid interpretation.

If standard deviations were not reported, we calculated them from the standard error and the number of replicates. Alternatively, we estimated them using the coefficient of variation from all complete cases, following the method described by Bracken (1992). Estimation of standard deviations was performed using the R package *metagear* (Lajeunesse, 2016; Terrer *et al.*, 2021). We also calculated the ratio of oxidative to hydrolytic C-degrading EEAs, the ratio of soil microbial C to N, and the fungal to bacterial biomass ratio. We employed the error propagation method (Lorber, 1986) to calculate the standard deviation (SD_y) of these ratios using Equation (3), where $\overline{r}$ and $\overline{s}$ are the mean values of *r* and *s*, respectively and SD_r and SD_s are the standard deviations, respectively.

$$\mathrm{SD}_y = \frac{\bar{r}}{\bar{s}} \times \sqrt{\left(\frac{\mathrm{SD}_r}{r}\right)^2 + \left(\frac{\mathrm{SD}_s}{s}\right)^2} \qquad (3)$$

The oxidative:hydrolytic C-degrading EEA ratio is an effective indicator for microbial substrate preference (Romero-Olivares, Allison & Treseder, 2017; Sinsabaugh, 2010; Yang *et al.*, 2019), with higher ratios indicating relatively greater investment in the decomposition of chemically recalcitrant C pools (Ren *et al.*, 2017; Romero-Olivares *et al.*, 2017).

Publication bias was examined using Begg’s test and Egger’s test (Begg & Mazumdar, 1994, 1994; Egger *et al.*, 1997; Leimu & Koricheva, 2004). Begg & Mazumdar (1994) proposed testing the interdependence of variance and effect size using Kendall’s method, and Egger et al. (1997) proposed a test for asymmetry based on a funnel plot. Our results showed that most variables did not have publication bias, and where there was potential publication bias for some variables, this would not affect the results because Rosenthal’s fail-safe number was much greater than 5*n*+10 (where *n* is number of observations; Table S1).

We examined the relative influence of multiple controlling factors on the responses of SOC storage, hydrolytic C-degrading EEAs, and oxidative C-degrading EEAs to N addition using model-selection analysis. We used the ‘*rma.mv ()*’ function from the R package *metafor* (Viechtbauer, 2010) and the ‘*glmulti()*’ function from the *glmulti* R package (Calcagno & de

Mazancourt, 2010) to automate the fitting of all possible models containing the predictors. We utilized the Akaike information criterion to select the most parsimonious models with the lowest Akaike value. The relative importance of a given predictor was ascertained by summing the Akaike weights, representing the probability that a particular model is the most plausible, for all models in which the predictor was present. A cut-off of 0.8 was set to differentiate between essential and non-essential predictor variables (Calcagno & de Mazancourt, 2010). We used '*lmer*' function in the *lme4* package to conduct linear mixed-effects models based on a restricted maximum likelihood approach (Bates *et al.*, 2015) to test the overall effects of significant predictors in Model Selection Analysis, as well as their interactions. To explore the possible mechanisms of changes in SOC storage under the condition of N addition, we also conducted meta-regressions between the RRs of SOC storage and the response of C-degrading EEAs, initial SOC content, N addition rate, and the ratio of N addition rate to background N deposition rate. We used Spearman's rank correlation analysis to evaluate the relationships of C-degrading EEAs with environment properties, soil properties, and N addition properties.

Based on known relationships, we fitted the data to a structural equation model to evaluate the direct and indirect effects of N addition on SOC storage. The model was fitted by maximum likelihood estimation in R using the *lavaan* package (Rosseel, 2012). The fit of the final model was evaluated using a chi-squared test. Models are considered to have a good fit when $0 \leq$ chi-squared/df ≤ 2, as well as when $0.05 < P \leq 1.00$ (Schermelleh-Engel, Moosbrugger & Müller, 2003).

III. RESULTS

(1) Responses of hydrolytic and oxidative C-degrading EEAs to N addition

Averaged across all studies, N addition had contrasting effects on hydrolytic and oxidative C-degrading EEAs (Fig. 2). Nitrogen addition significantly increased hydrolytic C-degrading EEAs by 12.8% with a range of 7.5% to 18.4%. This increase was primarily due to an increase in activities of β-1,4-glucosidase and β-1,4-D-cellobiohydrolase by 16.7% and 10.9%, respectively. By contrast, N addition significantly decreased oxidative C-degrading EEAs by 11.9% (ranging from –17.6% to –5.9%), due to a decrease in peroxidase, phenol oxidase and polyphenol oxidase activity by 7.8%, 14.6%, and 14.0%, respectively (Fig. 2C). Model selection analysis suggested that the responses of hydrolytic C-degrading EEAs to N addition were mostly explained by the initial SOC, N addition rate, and the climatic parameters MAT and MAP (Fig. 3B). In particular, the response of hydrolytic C-degrading EEAs to N addition was greater when initial SOC levels were high (Fig. S1B). By contrast, oxidative C-degrading EEAs were mostly explained by ecosystem type, soil type, and MAT (Fig. 3C). Specifically, N addition decreased oxidative C-degrading EEAs by 12.1% for both forest and grassland ecosystems (Fig. 4C). The responses of oxidative:hydrolytic C-degrading EEA ratio were mostly explained by ecosystem type, initial SOC, and N addition rate (Fig. 3D). N addition decreased the oxidative:hydrolytic C-degrading EEA ratio by 19.4% in forests and by 29.1% in farmland (Fig. 4D). In addition, N addition had more pronounced negative effects on oxidative:hydrolytic C-degrading EEA ratio when soils were C-rich (Fig. S1D) or when N addition rates were high (Fig. S2D). There were no significant relationships between the ratio of N addition rate to background N deposition rate (BND) and any of the RRs considered (Fig. S3).

(2) Linking shifts in C-degrading EEAs to changes in SOC storage with N addition

For studies that reported changes in SOC storage, N addition enhanced SOC storage by an average of 7.2% and increased soil recalcitrant SOC by 16.2% (Fig. 2B). The N addition-induced increases in SOC storage were predicted by ecosystem type, N-induced changes in oxidative C-degrading EEAs, and N addition rate (Fig.3A). Furthermore, we found that the response of SOC storage to N addition was positively related to hydrolytic and negatively correlated with oxidative C-degrading EEAs (Fig. 5A, B). The relationships between N-induced changes in C-degrading EEAs and SOC storage were significant over a variety of ecosystems and soil types (Figs 5 and S4). As a result, we found a strong negative relationship between the overall response of SOC storage and the responses of oxidative:hydrolytic C-degrading EEA ratio to N addition (Fig. 5C). According to the structural equation model, N-induced changes in SOC storage and oxidative C-degrading EEAs were strongly mediated by MBC rather than by changes in the ratio of fungal to bacterial biomass (Fig. 6). Specifically, reductions in MBC following N addition negatively affected oxidative C-degrading EEAs ultimately resulting in enhanced SOC storage. In addition, soil pH had a direct and significant effect on MBC, suggesting that N-induced soil acidification suppressed the decomposition of SOC by limiting the growth of soil microorganisms (Fig. 6).

(3) Effect of ecosystem type on N addition-induced changes in SOC storage and EEAs

Ecosystem type was identified as an important predictor for N addition-induced changes in SOC storage and C-degrading EEAs (Fig. 4A). Overall, N addition increased SOC storage by 7.0% in forests and by 15.1% in farmlands while having no discernible effect on SOC storage in grasslands and shrublands. Regarding hydrolytic C-degrading EEAs, N addition resulted in an increase of 13.3% in forests and 27.2% in farmlands (Fig. 4B). Conversely, we observed a reduction of 12.1% in oxidative C-degrading EEAs in both forests and grasslands following N addition (Fig. 4C). Moreover, changes in SOC storage induced by N addition were significantly related to the responses of oxidative C-degrading EEAs in all ecosystems (Fig. 5E) while a significant relationship between the response of SOC storage and the responses of hydrolytic C-degrading EEAs was only observed for forest ecosystems (Fig. 5D). In farmland ecosystems, our analysis revealed that the responses of hydrolytic C-degrading EEAs were negatively correlated with soil clay content and N addition duration (Fig. 4E). The responses of oxidative C-degrading EEAs were positively correlated with background soil total N content and clay content, and negatively correlated with background soil C:N ratio and N addition frequency. In forest ecosystems, significant correlations were observed between C-degrading EEAs and soil properties. Specifically, hydrolytic C-degrading EEAs showed a positive correlation with initial SOC and total nitrogen (N) content. In contrast, oxidative C-degrading EEAs exhibited negative correlations with these properties (Fig. 4E).

IV. DISCUSSION

(1) New insights into SOC dynamics under N addition from EEAs

Our investigation provides compelling evidence that N addition can significantly influence soil microbial anabolism, as manifested by alterations in C-degrading EEAs. Our analysis demonstrated that N addition augmented hydrolytic C-degrading EEAs while suppressing oxidative C-degrading EEAs (Fig. 2). These results are consistent with several recent studies that also reported these same patterns (Chen *et al.*, 2017; Jian *et al.*, 2016; Xiao *et al.*, 2018). N limitation can stimulate the production of oxidase enzymes, as N-containing molecules are often chemically bound within complex, recalcitrant substrates (Kuzyakov & Xu, 2013). By reducing microbial N limitation and increasing relative microbial C limitation with N addition (Sinsabaugh, 2010), hydrolytic C-degrading EEAs may be stimulated while oxidative C-degrading EEAs are suppressed. While a previous study identified experimental duration to be a crucial factor influencing the outcomes of C dynamics in plant and soil responses to N addition (Xu *et al.*, 2021), in our analyses these relationships between changes in C-degrading EEAs and SOC storage were unaffected by experimental duration (Fig. S5). This suggests that the strategies for using carbon employed by soil microbes remain consistent across different durations of N addition (Leff *et al.*, 2015). These robust relationships offer fresh perspectives into the mechanisms that regulate SOC storage in response to N addition.

N addition can alter soil C-degrading EEAs, thereby influencing both SOC decomposition and sequestration (Cao *et al.*, 2021; Riggs *et al.*, 2015). Microorganisms produce oxidative enzymes in part to mineralize structurally complex C sources to obtain N, which is often protected or shielded by recalcitrant substrates such as lignin (Manzoni *et al.*, 2012). Thus, the reduction in oxidative C-degrading EEAs and an increase in hydrolytic C-degrading EEAs may increase mineral-associated organic matter derived from the decay of cellulose (Margida *et al.*, 2020). This shift could increase the accumulation of microbial products and the formation of stable organic matter (Cotrufo *et al.*, 2013). This could also explain the significant increase in recalcitrant SOC under N addition (Fig. 2) and aligns with recent comprehensive meta-analyses of N-induced changes in recalcitrant soil carbon (Qi *et al.*, 2023; Xu *et al.*, 2024). Indeed, Chen *et al.* (2018*b*) showed that suppression of oxidative C-degrading EEAs by N addition exerted greater control over SOC storage than climatic and edaphic factors. A novel perspective acknowledges the substantial and direct impact of microbial biomass in the accumulation of organic matter. This process involves the transformation of microbial necromass into stable fractions of SOC (Kallenbach, Frey & Grandy, 2016; Ye *et al.*, 2018). For example, Lu *et al.* (2021*a*) reported an increase in soil aggregate formation and SOC sequestration under N addition. Finally, increased SOC storage under N addition may result from decreased microbial N mining from recalcitrant SOC, known as a negative priming effect (Fanin, Alavoine & Bertrand, 2020; Hicks, Lajtha & Rousk, 2021). In the presence of excess N, soil microbes switch to using exogenous N sources rather than degrading SOC for N (Allison & Vitousek, 2005). The reduced 'mining' for organic nutrients can conserve organic matter and result in greater SOC storage (Blagodatskaya *et al.*, 2007; Nottingham *et al.*, 2015).

In summary, N addition can have multifaceted effects on SOC storage through its impact on microbial growth and EEAs. The equilibrium between oxidative and hydrolytic C-degrading EEAs plays a pivotal role in determining the net effect of N addition on SOC storage. Further research is imperative to advance understanding of the mechanisms underlying these effects and to devise strategies for managing N addition to enhance SOC storage.

(2) Factors affecting the enzyme control of SOC storage under N addition

We provide a framework (Fig. 6) proposing several factors modulating the enzymatic regulation of SOC storage under N addition. First, our data demonstrate that N addition significantly stimulated plant productivity (Fig. 2), but this increased plant productivity did not directly influence SOC storage (Fig. 6). Although previous studies have demonstrated that N addition significantly increased the aboveground productivity of various terrestrial ecosystems (Čapek *et al.*, 2018; Schulte-Uebbing & de Vries, 2018), our results suggest that these new C inputs do not necessarily increase SOC storage (Cotrufo *et al.*, 2015; Niu *et al.*, 2016; Lu *et al.*, 2021*a*). This is particularly true because greater C inputs coupled with more favourable soil C:N ratios, may accelerate microbial metabolic activity and SOC decomposition (Liang *et al.*, 2017). Consequently, an increase in plant biomass does not result systematically in an increase in SOC storage, and N addition can also have neutral or even negative effects on SOC storage depending on the specific driving mechanisms

(Crowther *et al.*, 2019; Liu & Greaver, 2010; Lu *et al.*, 2022). These negative responses can be ascribed to either N saturation over time or the accumulated deleterious effects of long-term N addition on plant and microbial growth (Treseder, 2008). Therefore, stimulated plant productivity alone cannot be considered as the sole determinant of alterations in SOC storage in terrestrial ecosystems (Xu *et al.*, 2021), with other mechanisms related to microbial functions remaining largely unexplored.

Changes in microbial community size and composition may contribute to changes in hydrolytic and oxidative C-degrading EEAs, resulting in changes to SOC storage. This is because individual microbial taxa produce only a subset of the enzymes required to degrade complex organic matter (Condron *et al.*, 2010). While many kinds of soil microorganisms can secrete hydrolytic C-degrading enzymes, only a small number of microorganisms produce oxidative C-degrading enzymes, such as white-rot basidiomycetes and xylacarious ascomycetes (Carreiro *et al.*, 2000). N addition often results in a decrease in fungal biomass and an increase in bacterial biomass (He, Ruan & Jia, 2024; Huang *et al.*, 2023). With a lower fungi-to-bacteria ratio, the soil's efficiency in forming stable SOC is reduced, potentially diminishing long-term SOC storage (Ali *et al.*, 2021). Fungi and bacteria perform distinct roles in SOC storage. Bacteria typically decompose organic matter more rapidly, leading to faster SOC turnover and less stable SOC storage (Don *et al.*, 2017). Conversely, fungi are more efficient at decomposing complex organic materials and forming stable soil aggregates, which enhances SOC storage (Fan *et al.*, 2022). Mycorrhizal fungi, in particular, produce a variety of EEAs that aid in organic matter decomposition (Parihar *et al.*, 2020). They initially stimulate the decomposition of fresh residues and later tend to suppress the decomposition of older or more decomposed SOC (Wei *et al.*, 2019). The extensive hyphal networks of these fungi contribute to SOC by transporting carbon from plants to soil and forming stable soil aggregates (Wang *et al.*, 2023; Kohler *et al.*, 2017). Saprophytic fungi are the primary cellulose degraders, while mycorrhizae (including many basidiomycetes and ascomycetes) likely function as recalcitrant nitrogen miners. Augmented by their plant hosts' energy budgets, these mycorrhizae are also the main producers of phenol oxidase (Burke & Cairney, 2002). Additionally, N addition promotes SOC accumulation by enhancing the relative contribution of roots compared to hyphae in SOC accrual (Yuan *et al.*, 2024). Moreover, previous studies found that N addition can reduce microbial biomass by 5–20% (Lu *et al.*, 2011; Xiao *et al.*, 2018; Zhang, Chen & Ruan, 2018), possibly contributing to shifts in taxonomic composition. Our analysis revealed positive associations between oxidative C-degrading EEAs (but not hydrolytic EEAs) and MBC under N addition (Table S3), demonstrating that increasing total microbial biomass drives the increase in soil oxidative C-degrading EEAs (Fig. 6). These results suggest that shifts in C-degrading EEAs with N addition were associated with changes in microbial community composition. However, the underlying mechanism of this response remains unclear. High-resolution analyses that provide more detailed taxonomic information are necessary to assess the structural response of soil microbial communities to N addition.

Finally, N-induced alterations in soil pH could contribute to enzymatic control of SOC storage. Soil pH affects the binding of substrates to enzymes and the formation of enzyme proteins (Sinsabaugh, 2010). Our shifts in C-degrading EEAs with the reduction in soil pH are consistent with previous explanations for changes in SOC accumulation under N addition due to changes in microbial biomass and activities (Funakawa *et al.*, 2014). We found that N addition-induced soil acidification (Fig. 2) inhibited microbial growth and oxidative C-degrading EEAs, which were accompanied by decreased MBC (Fig. 6), microbial respiration and SOC decomposition (Averill & Waring, 2018; Janssens *et al.*, 2010; Niu *et al.*, 2016; Wang *et al.*, 2018). Moreover, the consistently negative effect of high soil pH on SOC storage suggests that low soil pH may increase the capacity for SOC storage and nutrient supply in specific biome types (Chen *et al.*, 2018*c*).

In summary, the responses of soil C-degrading EEAs to N addition are complex. The net effect depends on how both enzyme production and turnover are affected by changes in both soil environment and resource availability. Enzyme synthesis and secretion in natural environments are influenced by a combination of abiotic factors such as soil pH and temperature, as well as biotic processes including the composition of the soil microbial community and root–microbe interactions. These abiotic and biotic factors, however, can vary across different ecosystem types. Consequently, EEAs are likely to be responsive to N addition across various ecosystem types, as well as to other climate changes (Chen *et al.*, 2018*b*, 2020*a*). These changes will have important consequences for ecosystem functions such as decomposition and nutrient cycling, which will ultimately affect SOC storage.

(3) Uncertainties and implications

Our findings provide novel insights into the relationships between N addition, soil extracellular EEAs, microbial communities and SOC dynamics, however several uncertainties warrant further investigation. First, atmospheric N deposition involves low-dose and continuous N inputs into ecosystems, whereas many N addition experiments used only single pulses. High-dose inputs of N may elicit stronger short-term effects on SOC storage than N applied in several doses across the year (Cao *et al.*, 2020). Therefore, it is necessary to assess N addition at lower levels over extended timeframes. In addition, the N addition rate in most experiments exceeded background N deposition rates, and our results may therefore overestimate the responses of SOC storage to N addition (Fornara & Tilman, 2012). In our analyses, we computed the ratio of the N addition rate to the background N deposition rate (Rate:BND). This ratio serves as a measure of the additional N introduced into an environment compared to the naturally deposited amount. Although this ratio did not contribute significantly to explaining the responses of SOC storage or C-degrading EEAs to N addition (Fig. 3) and the relationships were not significant (Fig. S3), it is important to note that specific impacts can vary. These variations depend on ecosystem type and the form of N being added. Therefore, this ratio should be used in conjunction with other measurements and observations to comprehend fully the effects of N deposition. Second, the data set in this study was predominantly from Asia (Fig. 1), but geological differences among sites were not fully considered. Future studies should aim to include a wider range of geographically diverse samples to investigate the effects of geological influences. This would provide a more comprehensive understanding of the interplay between geology, soil microbes, and EEAs, enhancing our ability to predict and manage soil health and function under changing environmental conditions. Third, different responses of hydrolytic and oxidative C-degrading EEAs to N addition revealed divergent microbial nutrient acquisition strategies that regulate SOC storage, but it remains challenging to link the response of C-degrading EEAs to microbial community structure and diversity. Nitrogen addition often induces changes in microbial community structure that are consistent with changes in EEAs (Waldrop, Zak & Sinsabaugh, 2004*a*). As C mineralization is a complex process that involves the enzyme-mediated catalysis of various organic fractions (Wu *et al.*, 2022), the presumption of inflexible microbial functional traits during N addition may lead to inaccurate predictions of SOC storage. Although this meta-data synthesis establishes a framework for plant–soil–microbe interactions, future studies using new methods and technologies, such as advanced genome sequencing and probe-based technologies, remain necessary to predict accurately the overall consequences of N addition on SOC storage. In addition, given the strong enzymatic control of SOC storage under N addition, explicitly representing soil enzymatic processes as an important component of land surface models will improve our ability to generate a more realistic simulation of the responses of SOC storage to environmental changes (Zhang *et al.*, 2022).

V. CONCLUSIONS

(1) Based on a comprehensive global meta-data synthesis, we offer novel insights into the role of soil extracellular enzymes in mediating the response of SOC storage to N addition.

(2) Our analysis demonstrates that N addition significantly enhanced SOC storage, with this enhancement strongly correlated with shifts in soil C-degrading EEAs. Specifically, we observed an increase in hydrolytic C-degrading EEAs and a decrease in oxidative C-degrading EEAs following N addition, suggesting that soil microorganisms modulate the cycling of different C pools through distinct mechanisms.

(3) The net effect of N addition on SOC storage is determined by the balance between these two types of C-degrading EEAs, with impacts varying across different ecosystem types.

(4) These results highlight the profound effects of N addition on microbially mediated SOC storage in terrestrial ecosystems, emphasizing the importance of understanding these processes to predict SOC storage better under future climate change scenarios.

VI. ACKNOWLEDGEMENTS

We sincerely thank all the scientists whose data and studies were included in this meta-data synthesis. We also appreciate the insightful and constructive comments from the editor and anonymous reviewers that contributed to improving the manuscript. This study was financially supported by the National Natural Science Foundation of China (Grant number 42205167). J.Ch. is funded by the National Natural Science Foundation of China (32471685, 42361144886) and Shanxi Province Natural Science Foundation for Distinguished Young

Scholar (2024JC-JCQN-32). A.T.N. is supported by a UK Natural Environment Research Council grant (NE/T012226).

VII. DATA ACCESSIBILITY STATEMENT

All data needed to evaluate the conclusions in the paper are present in the paper and/or the supplementary materials and are also available at Figshare (https://figshare.com/s/41394218cf59d54b6608). Requests for additional materials and databases should be addressed to Ji Chen (chenji@ieecas.cn) or Xiao Chen (chenxiao@mail.iap.ac.cn).

VIII. AUTHOR CONTRIBUTIONS

J.Ch. and J.Ca. designed the research; X.C. performed the research, analysed data and wrote the paper; and R.L.S., D.L.M., R.D.B., N.F., A.T.N., J.Ch. and X.Z. contributed substantially to revisions.

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X. SUPPORTING INFORMATION

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

Appendix S1. PRISMA flowchart for assessment of eligible studies.

Appendix S2. List of included studies.

Table S1. Results for publication bias using Begg’s test and Egger’s test.

Table S2. Evaluation of model parameters used to explain SOC storage under N addition.

Table S3. Evaluation of model parameters used to explain C-degrading extracellular enzyme activities (EEAs) under N addition.

Table S4. Summary of mixed effect models of significant predictors in Model Selection Analysis, as well as their interactions on the response of SOC storage and C-degrading EEAs to nitrogen addition.

Fig. S1. Relationship between initial soil organic carbon (SOC) content and the response ratios of SOC storage, hydrolytic C-degrading extracellular enzyme activities (EEAs), oxidative C-degrading EEAs, and oxidative:hydrolytic C-degrading EEA ratio to N addition.

Fig. S2. Relationship between N addition rate and the response ratios of soil organic carbon (SOC) storage, hydrolytic C-degrading extracellular enzyme activities (EEAs), oxidative C-degrading EEAs, and oxidative:hydrolytic C-degrading EEA ratio to N addition.

Fig. S3. Relationship between N addition rate:background N deposition rate (Rate:BND) and the response ratios of soil organic carbon (SOC) storage, hydrolytic C-degrading extracellular enzyme activities (EEAs), oxidative C-degrading EEAs, and oxidative:hydrolytic C-degrading EEA ratio to N addition.

Fig. S4. Relationships between the response ratios (RRs) of soil organic carbon (SOC) storage and extracellular enzyme activities (EEAs) to N addition for studies categorized by soil type.

Fig. S5. Effect of experimental duration on the relationship between the response of soil carbon storage and C-degrading extracellular enzyme activities (EEAs) under N addition.

Figure S6 Relationship between the responses ratio of SOC storage to N addition and the responses of hydrolytic C-degrading EEAs, oxidative C-degrading EEAs, and oxidative:hydrolytic C-degrading EEA ratio to N addition in natural ecosystems (forest, grassland and shrubland).

Figure S7 Relationships between the response ratios of pH to N addition (RR-pH) and the difference in soil pH between N addition and control treatments (ΔpH).

Figure S8 Meta-data synthesis of the average effects (%) of N addition on SOC storage, hydrolytic C-degrading EEAs, oxidative C-degrading EEAs, and oxidative:hydrolytic C-degrading EEA ratio for N addition properties.

Figure S9 The distribution of relevant studies published over the past several years.

Table 1. Overview of the enzymes included in our meta-data synthesis. EC is the enzyme commission number classification for that enzyme or class of enzymes.

Type	**Extracellular enzyme**	**EC**	**Target**
Hydrolytic	β-1,4-glucosidase	3.2.1.21	Cellulose degradation products
	β-1,4-xylosidase	3.2.1.37	Hemicellulose degradation products
	β-D-cellobiosidase	3.2.1.91	Cellulose degradation products
Oxidative	Peroxidase	1.11.1.7	Lignin and other complex compounds
	Phenol oxidase	1.10.3.2	Lignin and other complex compounds
	Polyphenol oxidase	1.14.18.1	Lignin and other complex compounds

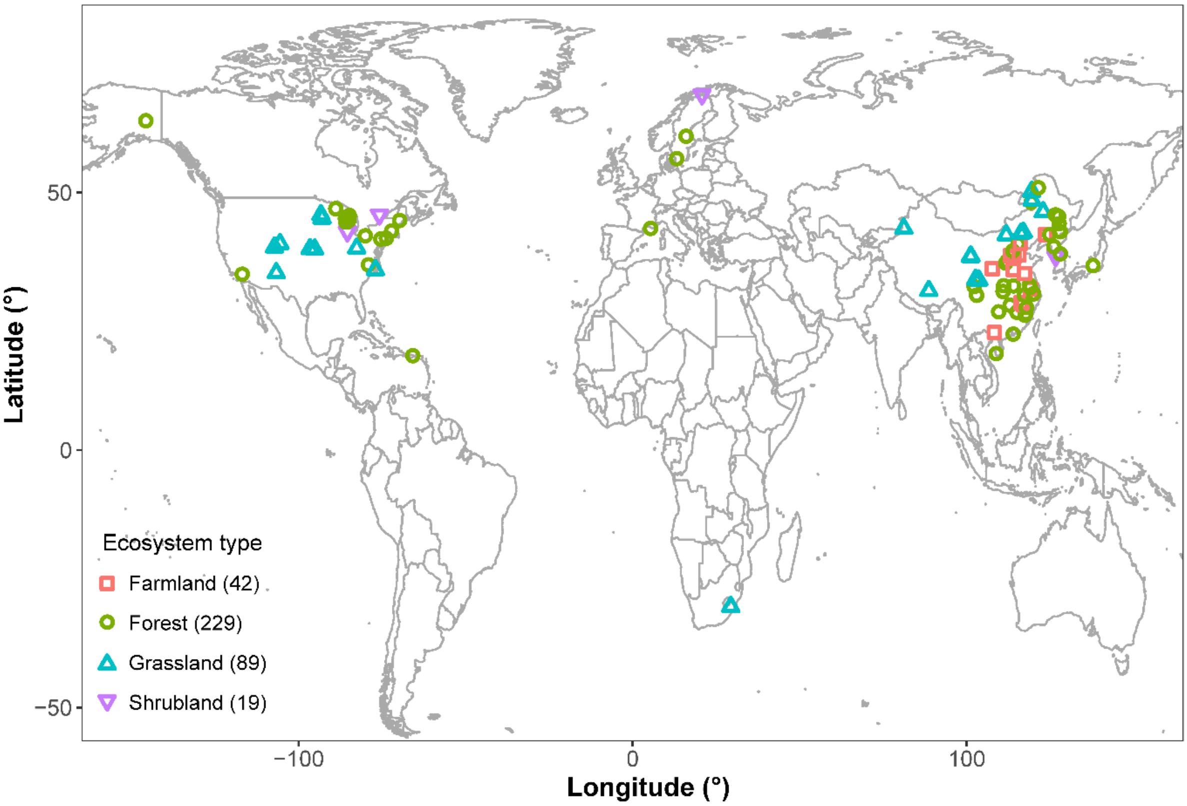

Fig. 1. Global distribution of the sites included in this meta-data synthesis. The numbers in parentheses in the key are the number of observations for each ecosystem type.

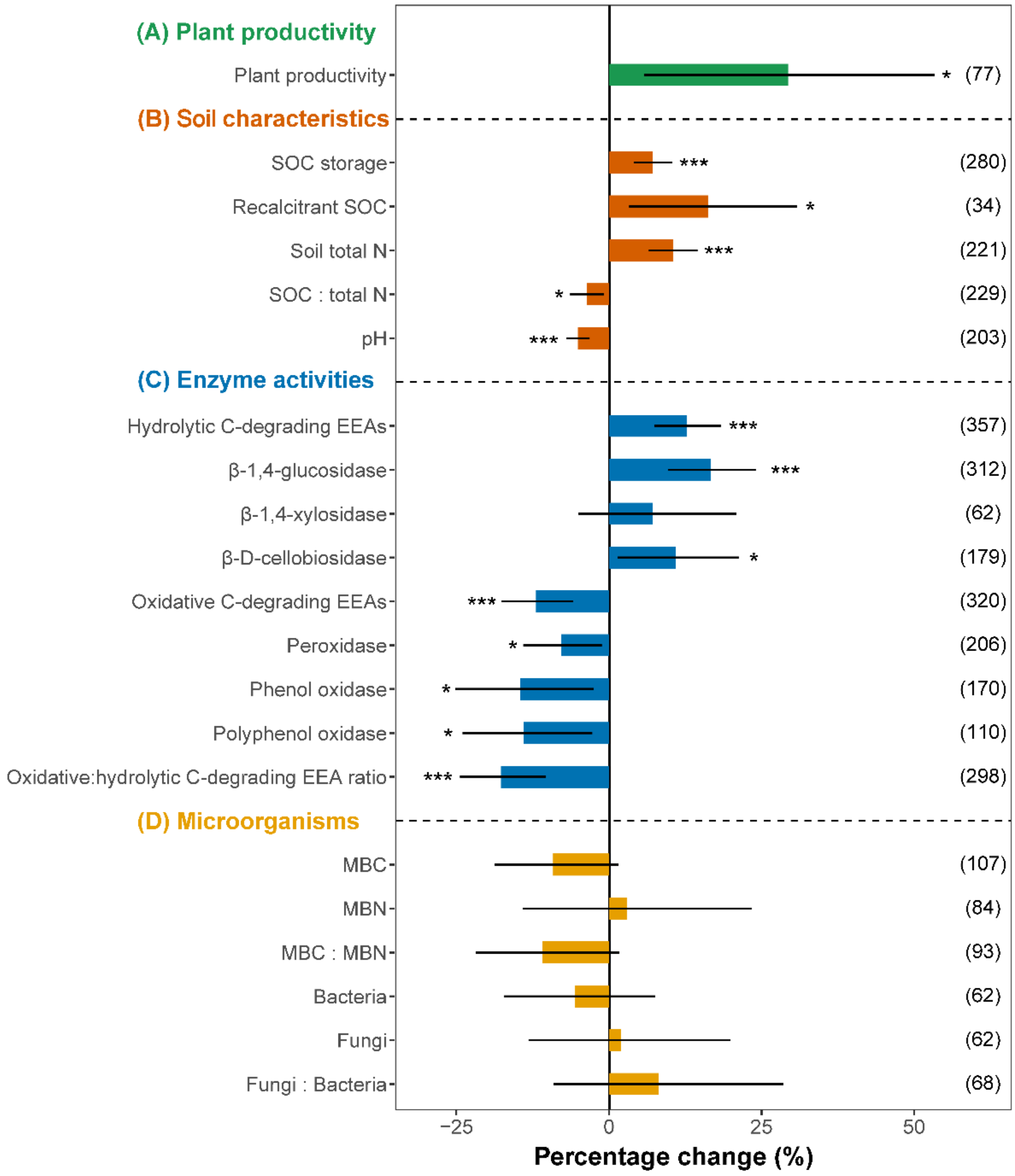

Fig. 2. Results of meta-data synthesis of the average effects (%) of N addition on (A) plant productivity, (B) soil characteristics, (C) C-degrading extracellular enzyme activities (EEAs), and (D) microorganismal biomass. Error bars represent 95% confidence intervals. Asterisks indicate statistical significance: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. The vertical solid grey line is the zero line, and the numbers on the right of each panel indicate the sample size. MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; Soil N, soil total nitrogen; SOC, soil organic carbon.

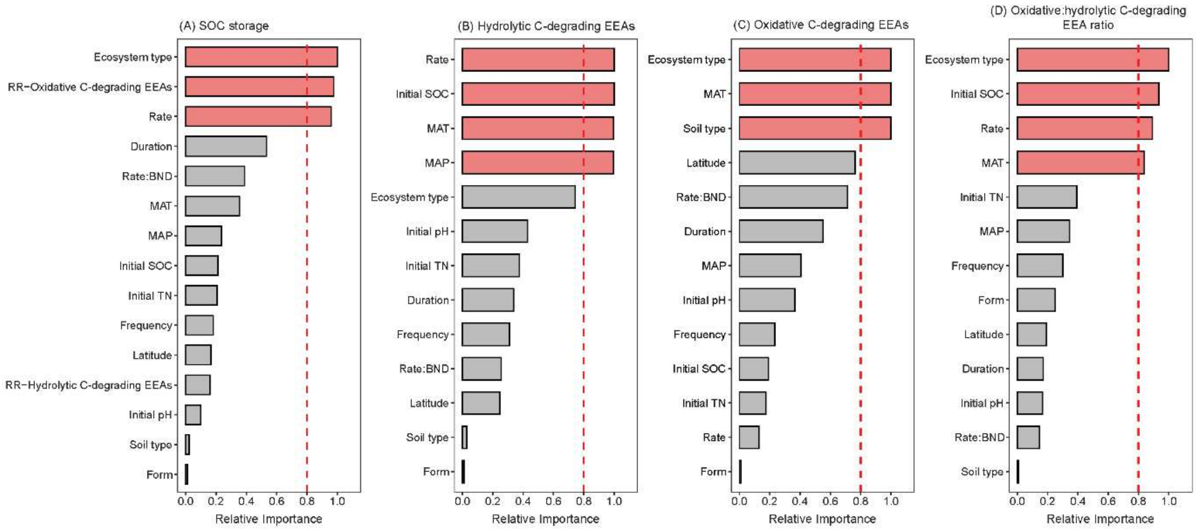

Fig. 3. Model-averaged importance of the predictors of the effects of N addition on (A) soil organic carbon (SOC) storage, (B) hydrolytic C-degrading extracellular enzyme activities (EEAs), (C) oxidative C-degrading EEAs, and (D) oxidative:hydrolytic C-degrading EEA ratio. The importance value is based on the sum of the Akaike weights derived from the model selection using corrected Akaike's information criteria. The cut-off was set at 0.8 to explore the most important variables. In this analysis, duration (year) was treated as a numeric variable. BND: background N deposition rate (g m^{-2} yr^{-1}); MAP: mean annual precipitation; MAT: mean annual temperature; RR, response ratio; TN, total nitrogen. Ecosystem type: farmland, forest, grassland, and shrubland; Soil type: Alfisol, Aridisol, Entisol, Gelisol, Histosol, Inceptisol, Mollisol, Oxisol, Spodosol, Ultisol; Rate: N addition rate (g m^{-2} yr^{-1}); Form: NH_4^+-N, NO_3^--N, NH_4NO_3, urea, and mixed fertilizer.

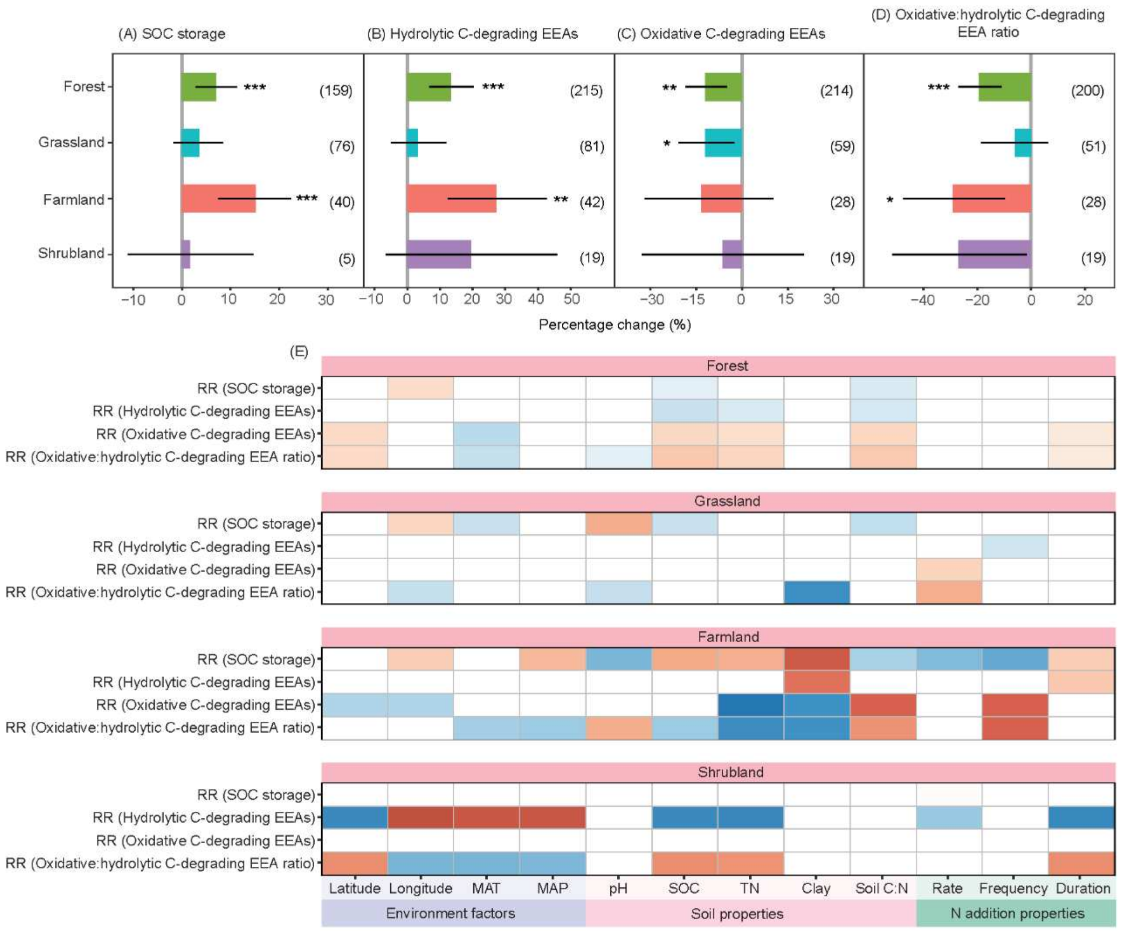

Fig. 4. Average effects (%) of N addition on (A) soil organic carbon (SOC) storage, (B) hydrolytic C-degrading extracellular enzyme activities (EEAs), (C) oxidative C-degrading EEAs, and (D) oxidative:hydrolytic C-degrading EEA ratio for various ecosystem types. The error bars represent the 95% confidence intervals. Asterisks indicate statistical significance: * P < 0.05; ** P < 0.01; *** P < 0.001. The vertical solid grey line is the zero line, and the numbers on the right of each panel indicate the sample size. (E) Relationships between different variables and the response ratio (RR) of SOC storage, hydrolytic C-degrading EEAs, oxidative C-degrading EEAs, and oxidative:hydrolytic C-degrading EEA ratio. MAP: mean annual precipitation; MAT: mean annual temperature; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; pH, background soil pH; SOC, background soil organic carbon; TN, background soil total nitrogen. Clay, background clay content expressed in %.

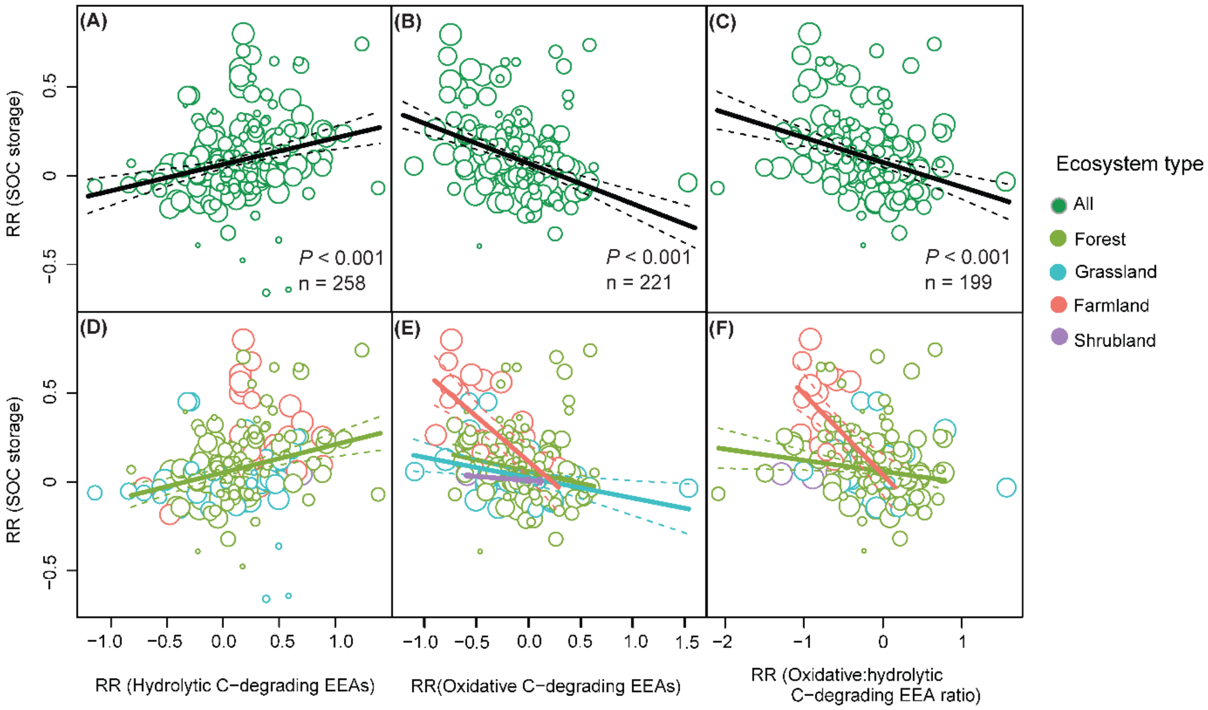

Fig. 5. Relationship between the response ratio (RR) of soil organic carbon (SOC) storage to N addition and the responses of hydrolytic C-degrading extracellular extracellular enzyme activities (EEAs) (A, D), oxidative C-degrading EEAs (B, E), and the ratio of oxidative to hydrolytic C-degrading EEAs (C, F) to N addition across various ecosystem types. The regression lines indicate a significant relationship across all ecosystems (A–C) and within each ecosystem type (D–F), as determined by a mixed-effects meta-regression model with a 95% confidence interval. The bubbles represent individual experiments included in the meta-data synthesis, with the size of the bubbles being proportional to the model weights.

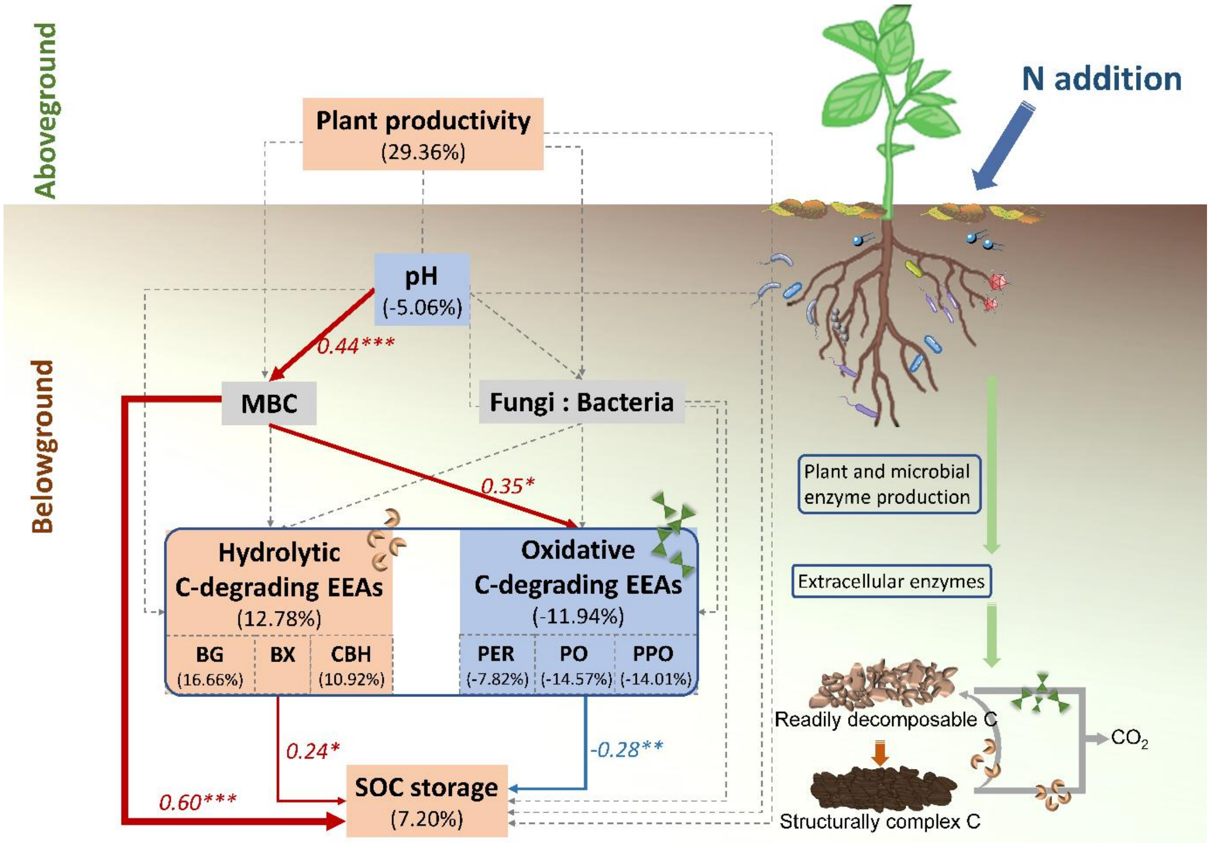

Fig. 6. Structural equation models investigating multivariate effects on soil organic carbon (SOC) storage under nitrogen addition ($\chi^2 = 7.39$, df = 4, $P = 0.12$; $R^2 = 0.36$). The orange and blue rectangles represent the significant positive and negative effects of N addition, respectively. The grey rectangles represent non-significant effects. Numbers in parentheses indicate the average effects (%) of N addition. Arrows indicate the hypothesized direction of causation, with red and blue arrows representing significantly positive and negative relationships, respectively. Grey dashed arrows indicate non-significant relationships. Numbers next to arrows are standardized path coefficients. BG, β-1,4-glucosidase; BX, β-1,4-xylosidase; CBH, β-D-cellobiosidase; EEA, extracellular enzyme activity; MBC, microbial biomass carbon; PER, peroxidase; PO, phenol oxidase; PPO, polyphenol oxidase. Fungi:bacteria is fungal:bacterial biomass ratio. Asterisks indicate statistical significance: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.