Mycorrhizal association controls soil carbon-degrading enzyme activities under nitrogen addition: Implications for soil carbon dynamics

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Abstract

Increasing atmospheric nitrogen (N) deposition affects soil carbon (C) dynamics in terrestrial ecosystems. Recent evidence suggests that changes in C-degrading extracellular enzyme activities (C-EEAs) largely explain the responses of soil C dynamics to N deposition. However, the factors that control C-EEA variations under N addition remain unclear, impeding the inclusion of microbial mechanisms in global C cycle models. Using a global meta-analysis, we show that across a wide range of experimental and environmental factors, the response of C-EEAs to N addition is best predicted by mycorrhizal association. In ecosystems dominated by ectomycorrhizal plants, N addition stimulated cellulase activity by 24.4%, suppressed ligninase activity by 13.8%, and increased soil C stock by 5.8% on average. Nitrogen addition did not affect C-EEAs and soil C pool in ecosystems dominated by arbuscular mycorrhizal plants. Incorporating mycorrhizae in global C cycle models could improve predictions of soil C storage and stabilization under N enrichment.

1. Introduction

Anthropogenic activities such as fossil-fuel combustion and the use of chemical fertilizer have greatly increased nitrogen (N) inputs into the terrestrial biosphere (Gruber & Galloway, 2008; Fowler et al., 2013; IPCC, 2021). Widespread N deposition strongly affects carbon (C) dynamics of terrestrial ecosystems, since the majority terrestrial ecosystems are primarily limited by N (Hu et al., 2001; Schimel & Weintraub 2003; LeBauer & Treseder 2008). Soil C stocks are determined by the balance between C input through plant growth and C losses through microbial decomposition. Added N may increase soil C storage by increasing plant growth and C input (LeBauer & Treseder, 2008; Reay et al., 2008). However, added N can also increase soil C storage even without stimulating plant growth (Mack et al., 2004; Knorr et al., 2005; Janssens et al., 2010), highlighting the importance of microbial decomposition in regulating soil C dynamics under N deposition.

Extracellular enzymes catalyze rate-limiting steps in microbial decomposition of soil organic matter (SOM), and the C-degrading extracellular enzyme activities (C-EEAs) play a critical role in regulating soil C dynamics (Schimel & Bennett, 2004; Jan et al., 2009; Chen et al., 2018a). The C-EEAs can be broadly classified as cellulase (e.g., β-1,4-glucosidase, β-1,4-xylosidase, and β-D-cellobiosidase) and ligninase (e.g., peroxidase, phenol oxidase, and polyphenol oxidase) (Margida et al., 2020). Cellulases can be synthesized by a wide group of microbes, whereas only a small number of microbes such as white-rot basidiomycetes and xylarcarious ascomycetes produce ligninases. Microbes prefer to produce cellulases that catalyze the hydrolysis of cellulose and polysaccharides to meet microbial N demand by making other N-rich labile substances (e.g., peptides) available for decomposition (Allison et al., 2008; Sinsabaugh et al., 2010). However, with the depletion of labile substrates and aggravated N limitation, microbial community composition may shift towards high abundance of fungi to produce ligninases that catalyze the oxidation of recalcitrant substrates, such as aromatic C polymers and lignin (Kuzyakov & Xu, 2013), despite consuming more energy. Nitrogen addition generally stimulates cellulase activity but suppresses ligninase activity (Jian et al., 2016), and these changes in C-EEAs are proposed as primary
controls of N impact on soil respiration and C storage (Chen et al., 2017, 2018b), providing a promising pathway to explore the role of microbes in soil C cycling. However, the underlying mechanisms associated with the responses of cellulase and ligninase to N addition remains unclear.

Most plant species on Earth form associations with mycorrhizal fungi, of which ectomycorrhizal (ECM) and arbuscular mycorrhizal (AM) fungi are the two most common types (Smith & Read, 2008). ECM fungi can produce a wide range of enzymes that degrade soil organic N compounds (Phillips et al., 2013; Lindahl & Tunlid, 2015), whereas AM fungi lack these enzyme systems and primarily take up inorganic N (Pellitier & Zak, 2018). The uptake of organic N via ECM fungi facilitates ECM plants to acquire substantially more N from the soil than do AM plants (Read & Perez-Moreno, 2003), directly competing with soil free-living microbial decomposers for organic N (Orwin et al., 2011). Moreover, compared to AM plants, litter from ECM plants (primarily wood plants) generally contain higher C:N ratios and greater concentrations of lignin and polyphenolic compounds (Lin et al., 2017). These specific N-acquisition strategy and leaf N economy of ECM plants often results in high C:N ratio of lignin-derived soil organic matter (SOM) and aggravates N limitation of soil free-living microbial decomposers particularly bacteria and their production of extracellular enzymes (Orwin et al., 2011; Phillips et al., 2013; Averill et al., 2014; Fernandez & Kennedy, 2016). However, recent progresses suggest that, in order to meet substantially higher N uptake and demand for ECM-associated than AM-associated plants, ECM fungi can produce ligninase to accelerate the decay of lignin-derived SOM (Sulman et al., 2017; Argiroff et al., 2021). Nitrogen addition can alleviate plant and microbial N limitation and the competition between ECM fungi and free-living microbial decomposers for organic N, which likely alters their production and type of extracellular enzymes and hence affect soil C storage (Averill et al., 2014). This suggests that mycorrhizal association may be a key factor determining the response of soil C-EEAs to N addition, with possible implications for soil C storage. However, this hypothesis has never been explicitly tested and little is known to what extent mycorrhizal association influences soil C storage under N addition.

We synthesized data for C-EEAs, including both cellulase and ligninase from 84 N addition experiments around the world (Table S1; Figure S1). Through meta-analysis, we investigated the role of a wide range of environmental and experimental factors in determining the response of C-EEAs to N addition. Mycorrhizal type (ECM-dominated vs. AM-dominated ecosystems), vegetation type (grassland vs. forest), mean annual temperature (MAT), mean annual precipitation (MAP), soil pH, and N addition methods (N addition form, rate, frequency and duration) of the experiments were considered as potential explanatory factors. The meta-analytic models were selected by using the same approach as in Terrer et al. (2016) and Chen et al. (2018b).

2. Results

Model selection analysis, based on the corrected Akaike Information Criterion, showed that over a broad range of environmental and experimental factors, the responses of both cellulase activity and ligninase activity to N addition were best predicted by mycorrhizal type (Fig. 1). Specifically, N addition significantly stimulated cellulase activity by 24.4% (95% CIs, 15.9% ~ 33.5%) and suppressed ligninase activity by
13.8% (8.1% ~ 19.2%) in ECM-dominated ecosystems, but it did not affect the activity of these two enzymes in AM-dominated ecosystems (Fig. 2). Furthermore, within this database, N addition significantly increased the size of the soil C pool by 5.8% (0.5% ~ 11.3%; Fig. 3a) and the recalcitrant C pool by 16.2% (5.7% ~ 27.8%; Figure S3) in ECM-dominated ecosystems, whereas no such effects were found in AM-dominated ecosystem. The response of SOC was best predicted by response of ligninase activity to N addition (Figure S4). Increases in soil C with N addition in ECM-dominated ecosystems were negatively correlated with the response of ligninase activity ($P < 0.05$), but did not correlate with the response of cellulase activity ($P = 0.82$) (Fig. 3b). In AM-dominated ecosystems, we found no significant relationships between the response of soil C and C-EEAs under N addition (Fig. 3c). Moreover, N addition significantly decreased soil pH by 0.14 units (0.02 ~ 0.26) in AM-dominated ecosystems, but it did not significantly affect soil pH in ECM-dominated ecosystems (Figure S5).

Herbs generally form mycorrhizal symbioses only with AM fungi (Brundrett, 2002; Bennett & Classen, 2020), while some woody trees can form symbiotic associations with either ECM fungi, AM fungi, or both (Table S1). To isolate the influence of mycorrhizal type from that of vegetation type, we ran a separate meta-analysis on the subset of N addition experiments for forest only. Within this subset, mycorrhizal type remained the most important predictor for the responses of C-EEAs to N addition; N addition stimulated cellulase activity and suppressed ligninase activity in ECM-dominated forest ecosystems, but not in AM-dominated forest ecosystems (Figure S6).

### 3. Discussion

Our meta-analysis shows that N addition significantly stimulates cellulase activity and suppresses ligninase activity in ECM-dominated ecosystems. ECM plants preferentially take up readily available N derived from new inputs and do not need to allocate more C to support N acquisition via ECM symbiosis (Pellitier et al., 2021). Thus, N addition likely suppresses ECM symbiosis and their production of ligninase (Corrales et al., 2017; Argiroff et al., 2021). Further, without the direct uptake of organic N by ECM fungi, more organic N should be locked up in SOM. Nitrogen addition enhances soil organic N storage and reduce soil C:N ratio (Nave et al., 2009; Lu et al., 2011). We speculate that this response may alleviate N limitation of soil free-living microbial decomposers and change their community composition from ligninase-associated fungi to cellulase-associated bacteria as observed by Freedman et al. (2015) and Zhang et al. (2018). For example, long-term experimental N addition in a pine forests (i.e., an ECM-dominated ecosystem) indeed showed a significant decrease in fungal biomass and fungal:bacterial biomass ratios, resulting in the stimulated cellulase activity but suppressed ligninase activity (Frey et al., 2004). Nitrogen addition may also influence C-EEAs through stimulating plant growth and litter inputs (Weintraub et al., 2013; Veres et al., 2015). However, because ECM plants can directly acquire organic N via ECM fungi to sustain their growth, N addition often has negligible and even negative effects on the growth of ECM plants (Boggs et al., 2005; Thomas et al., 2010; Ma et al., 2021). Indeed, a re-analysis of the dataset from a recent meta-analysis on the responses of plant growth to N addition (Xu et al., 2019) found no significant change in aboveground biomass in ECM plants under N addition (Figure S7).
Why did N addition not affect cellulase and ligninase activity in AM-dominated ecosystems? First, N addition may not significantly influence C-EEAs by changing the abundance of AM fungi because AM fungi often lack enzyme systems (Averill & Finzi, 2011; Phillips et al., 2013). Second, we propose that these results reflect the degree to which microbial N limitation is lifted by N addition. Without the competition like ECM with free-living microbial decomposers for organic N, microbial N limitation is less likely to occur in AM-dominated ecosystems (Averill et al., 2014). AM-dominated ecosystems are also generally characterized by relatively lower soil C:N ratios than ECM-dominated ecosystems do (Averill et al., 2014). Thus, N addition in AM-dominated ecosystems may have a relatively weak effect on SOM quality, and hence does not significantly affect C-EEAs (Phillips et al., 2013). Finally, compared with ECM-dominated ecosystems, N addition often stimulates the growth of AM plants and improves litter quality (Boggs et al., 2005; Thomas et al., 2010), which may stimulate microbial particularly bacteria activity and their production of cellulose because N-rich litter is more easily decomposed by bacteria than fungi (Weintraub et al., 2013; Song et al., 2017). However, under N addition, AM-dominated ecosystems have been reported to often have more NO$_3^-$ loss than ECM-dominated ecosystems (Midgley et al., 2014), and thus are more susceptible to soil acidification (Figure S5; MacDonald et al., 2002). Most bacteria adapt to neutral or slightly acidic soils, whereas fungi survive at relatively low soil pH (DeForest & Scott, 2010; Jones & Oburger, 2011). Thus, an N-induced soil acidification in AM-dominated ecosystem may inhibit microbial activity and change community composition with higher ratio of fungi to bacteria (Högberg et al., 2007), which may inhibit the production of cellulase. The balance of these factors is likely context-dependent, which together may explain why N addition had no significant effect on both cellulase and ligninase activities in the AM-dominated ecosystems.

Our findings indicate that mycorrhizal association largely determine the response of C-EEAs to N addition, across a broad range of climate factors, experimental N application methods and vegetation types (Fig. 1). Specifically, stimulated cellulase activity but suppressed ligninase activity in ECM-dominated ecosystems suggest reduced decomposition of recalcitrant C compared to an accelerated labile C pool under N addition (Figure S3). Ligninases have recently been identified as keystone microbial enzymes for N control of soil C storage (Chen et al., 2018b), because they catalyze the oxidation of structurally complex macromolecules which play a key role in soil C stabilization. Similarly, we show that soil C storage under N addition was negatively correlated with the response of ligninase activity, whereas it did not correlate with the response of cellulase activity (Fig. 3b). The results reported here may help explain why N addition increased soil C storage in ECM-dominated ecosystems even with only minor effects on plant growth (Fig. 3a; Boggs et al., 2005; Thomas et al., 2010).

These findings may have important implications for the development of soil C models. Previous studies suggested that an incorporation of the critical process of enzyme-mediated microbial decomposition may substantially improve soil C predictions in earth system models (ESMs) (Wieder et al., 2013; Chen et al., 2019, 2020). Our results further highlight the importance of mycorrhizal association in controlling C-EEAs under N addition. The differential sensitivities of C-EEAs under N addition between ECM- and AM-dominated ecosystems may imply a complex mechanism of plant-microbe interactions in enzyme-
mediated SOM decomposition, which are largely associated with functional differences between AM and ECM fungi. However, these differences have not yet been integrated into the ESMs, likely resulting in biased predictions of soil C dynamics in the future N deposition scenarios.

Our findings may also advance the understanding the responses of AM-dominated and ECM-dominated ecosystems to other global change factors. For instance, elevated CO$_2$ usually aggravates ecosystem N limitation (Luo et al., 2006). Our findings suggest that the greater N limitation under elevated CO$_2$ would likely stimulate ECM fungi to produce more ligninase that degrade organic N from lignin-derived SOM in order to maintain plant growth, thereby stimulating soil C losses in ECM-dominated ecosystems. This prediction is consistent with the fact that elevated CO$_2$ has limited impact on soil C storage and even decreased the recalcitrant C pool in ECM-dominated ecosystems under elevated CO$_2$, despite greater biomass gains in ECM than in AM plants (Terrer et al., 2016, 2021). Hence, explicitly incorporating the role of mycorrhizae in determining cellulase and ligninase activity into global C cycle models can improve our ability to predict how ongoing environmental change impacts soil C storage.

**Materials And Methods**

**Data collection**

We used the original dataset from Chen et al. (2018b), and updated this dataset before April 2022 by searching for peer-reviewed articles from Web of Science (http://apps.webofknowledge.com/), Google Scholar (http://scholar.google.com/), and China National Knowledge Infrastructure (http://www.cnki.net/). The keywords and phrases used for the article selection were: (a) “nitrogen addition” or “nitrogen fertilizer” or “nitrogen amendment” or “nitrogen enrichment” or “nitrogen elevated” or “nitrogen deposition”; (b) “glucosidase” or “cellobiosidase” or “xylosidase” or “cellulase”; (c) “peroxidase” or “phenol oxidase” or “polyphenol oxidase” or “lignin modifying enzymes”; (d) “terrestrial” or “soil” or “land”.

Articles had to meet the following requirements to be included in our dataset. First, we only considered field experiments in natural grasslands and forest ecosystems. We ignored data from croplands and other managed ecosystems, because disturbance through agricultural practices may alter plant-microbe interactions. Second, we only included studies that simultaneously measured at least one kind of cellulase and ligninase. Third, we only considered studies where the dominant mycorrhizal type at the experimental site could be clearly identified. The mycorrhizal type is identified based on a site-specific vegetation observation of dominant plant species (Soudzilovskaia et al., 2019; Terrer et al., 2021). Mycorrhizal classification in forest ecosystems did not consider information on understory species, as biomass of understory plants is quite small compared to canopy trees. Fourth, details on N addition methods (fertilization rate, frequency, form, and duration) had to be provided.

Observations were not included in our dataset if they met any of the following exclusion criteria: (1) duplicate reports of the same studies, ensuring the independence of each study; (2) C-EEAs observations
with soil sampling depth below 20 cm, because the strongest interaction between plants and microorganisms generally occurs in the topsoil; (3) N addition studies with inputs of other nutrients (e.g., P, K, Ca, compost or slurry additions) or other global change treatments (e.g., CO₂, warming or precipitation change). Finally, we selected 59 published articles including 84 independent field experiments of N addition (Fig. S1). We did not find significant publication bias, as the points in the funnel plot were roughly symmetrically distributed (Fig. S2).

To explore the underlying mechanisms related to the responses of cellulase and ligninase activities to N addition, we recorded mycorrhizal type (ECM-dominated ecosystems and AM-dominated ecosystems); environmental factors including climate zone (tropic and temperate), vegetation type (grassland and forest), mean annual temperature (MAT), mean annual precipitation (MAP) and soil pH; N addition methods, including fertilization rate, fertilization frequency, fertilization form, and fertilization duration. As N fertilization form, we considered NH₄, NO₃, NH₄NO₃ or urea addition, as well as the mixture of NH₄, NO₃ and urea. We obtained unreported MAT and MAP from the WorldClim database (www.worldclim.org/). We obtained unreported soil pH from Tomislav (2018) (https://zenodo.org/record/2525664). Unreported SD values were estimated as 1/10 of the means (Gattinger et al., 2012). Where available, we recorded the responses of the size of the soil C and recalcitrant C pools and soil pH. When results were presented graphically, we used GetData Graph Digitizer v.2.24 (http://getdata-graph-digitizer.com/) to digitize the data.

**Data analysis**

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

\[
\ln R = \ln \left( \frac{X_N}{X_C} \right) = \ln(X_N) - \ln(X_C) \tag{1}
\]

where \(X_C\) and \(X_N\) are mean of the studied variables in the ambient and N addition treatments, respectively. The effect sizes were weighted by the inverse of the pooled variance (\(V_i\))

\[
V_i = \frac{S_N^2}{n_N X_N^2} + \frac{S_C^2}{n_C X_C^2} \tag{2}
\]

where \(n_C\) and \(n_N\) are the replicate numbers, and \(S_C\) and \(S_N\) are the standard deviations in the ambient and N addition treatments, respectively.

We used mixed-effects models to evaluate the N addition on all response variables. The effects of “Study site” and “Publication” were considered as random effects since several study sites contributed more than one effect size (Chen et al., 2018b). The calculation was implemented through the “rma.mv” function from the R package “metafor” (Viechtbauer, 2010). Except for soil pH, results are reported as
percentage change to ease interpretation. The effects of N addition were considered significant if the 95% confidence interval (CI) did not overlap with zero.

The most important predictors of the effects of N addition on the activities of soil C-EEAs were selected by a meta-analytic model, following the approaches by Terrer et al. (2016) and van Groenigen et al. (2011). Briefly, the importance of each predictor was computed as the sum of Akaike weights for models that included this predictor, and 0.8 was set as a cutoff to identify the most significant predictors (Terrer et al., 2016). The analysis was implemented through the “glmulti” package in R (Calcagno & Mazancourt, 2010).

Declarations

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Availability of data and materials

The data generated or analyzed in this study are included in this article and its supplementary information files. Other materials that support the findings of this study are available from the corresponding author on reasonable request.

Author contributions

Chen J and Deng Q conceived the study; Hu Y, Chen J, Chen G and Deng Q collected the data; Hu Y, Chen J, Olesen JE, van Groenigen KJ and Deng Q collaborated on data synthesis and interpretation; Hu Y, Chen J and Deng Q wrote the first draft; Olesen JE, van Groenigen KJ, Hui D and He X contributed critically to the drafts. All authors edited the manuscript and gave final approval for submission.

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

References


57. Tomislav H. 2018. Soil pH in H2O at 6 standard depths (0, 10, 30, 60, 100 and 200 cm) at 250 m resolution (v0.2) [Dataset]. Zenodo.


Figures

Model selection analyses identified mycorrhiza type as the most important predictor of the responses of cellulase and ligninase activities to nitrogen addition (n = 152). The model-averaged importance of the predictors of the response of cellulase (a) and ligninase (b) activities to N addition. The importance is based on the sum of Akaike weights derived from model selection using AIC (Akaike's Information Criteria). Cut-off is set at 0.8 (dashed line) to differentiate important from non-essential predictors. N: nitrogen; MAT: mean annual temperature; MAP: mean annual precipitation; BND: background nitrogen deposition rates; Rate: N fertilization rate; Frequency: N fertilization frequency; Duration: N fertilization duration.

Figure 1
Figure 2

Meta-analysis of the effects of nitrogen addition on cellulase and ligninase activities when grouped by mycorrhizal types. Error bars represent bootstrap 95% confidence interval (CIs); the effects of nitrogen addition were considered significant if the 95% CIs did not overlap with zero; the numbers above the error bar indicate sample sizes. ECM: ectomycorrhizal; AM: arbuscular mycorrhiza.
Figure 3

Results of a meta-analysis on the response of soil organic carbon (SOC) to nitrogen addition and the relationships of the response ratios (ln $R$) of soil organic carbon to cellulase and ligninase activities. (a) The effects of nitrogen (N) addition on SOC when grouped by mycorrhizal types; error bars represent bootstrap 95% confidence interval (CIs); the effects of N addition were considered significant if the 95% CIs did not overlap with zero; the numbers above the error bar indicate sample sizes. (b) The light gray area indicates the confidence interval around the regression line; a negative relationship was found between the response of SOC (ln $R$-SOC) and the response of ligninase activities (ln $R$-ligninase) based on observed data at ECM-dominant ecosystem ($r^2 = 0.098, P < 0.05$); no significant relationship was found between ln $R$-SOC and ln $R$-ligninase based on observed data for AM-dominant ecosystem ($r^2 = 0.001, P = 0.82$). (c) No significant relationship was found between ln $R$-SOC and the response of cellulase activities (ln $R$-cellulase) based on observed data for AM-dominant ecosystem ($r^2 = 0.041, P = 0.23$) or AM-dominant ecosystem ($r^2 = 0.001, P = 0.87$). ECM: ectomycorrhizal; AM: arbuscular mycorrhiza.

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