ECOLOGY

A keystone microbial enzyme for nitrogen control of soil carbon storage

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Agricultural and industrial activities have increased atmospheric nitrogen (N) deposition to ecosystems worldwide. N deposition can stimulate plant growth and soil carbon (C) input, enhancing soil C storage. Changes in microbial decomposition could also influence soil C storage, yet this influence has been difficult to discern, partly because of the variable effects of added N on the microbial enzymes involved. We show, using meta-analysis, that added N reduced the activity of lignin-modifying enzymes (LMEs), and that this N-induced enzyme suppression was associated with increases in soil C. In contrast, N-induced changes in cellulase activity were unrelated to changes in soil C. Moreover, the effects of added soil N on LME activity accounted for more of the variation in responses of soil C than a wide range of other environmental and experimental factors. Our results suggest that, through responses of a single enzyme system to added N, soil microorganisms drive long-term changes in soil C accumulation. Incorporating this microbial influence on ecosystem biogeochemistry into Earth system models could improve predictions of ecosystem C dynamics.

INTRODUCTION

Terrestrial ecosystems worldwide have experienced unprecedented reactive nitrogen (N) deposition during the past decades, and future global N deposition is expected to increase by 2.5 times or more over this century (*1*–*3*). Enhanced N deposition has been suggested to increase soil carbon (C) storage (*2*–*7*) as N fertilization generally stimulates plant growth and thus C input to soil. However, N-stimulated C input may or may not lead to increased soil C storage depending on the responses of decomposition to N addition (*8*–*11*). In some cases, N addition has been shown to reduce soil C storage by enhancing decomposition, a response that can override the stimulating effect of N addition on plant growth (*8*, *10*, *12*). On the other hand, N fertilization can significantly increase soil C storage at N-rich sites, where N addition has minor effects on plant growth but suppresses decomposition (*4*, *13*).

Decomposition is catalyzed by microbially produced extracellular enzymes, which break down dead plant and microbial biomass, and depolymerize macromolecules (*14*–*16*). N addition can alter extracellular enzyme activity, suppressing the activity of lignin-modifying enzymes (LMEs; enzymes that catalyze the breakdown of chemically recalcitrant substrates) and enhancing cellulase activity (table S1) (*9*, *17*–*20*). These responses are apparent in short-term assays of enzyme activity and consistent across ecosystems (*19*, *21*, *22*), but how they translate to long-term changes in soil C in response to N input is unknown. Here, we tested the hypothesis that N-induced shifts in C-degrading extracellular enzyme activities control changes in soil C storage. We assembled a database of C-degrading enzyme activity and soil C storage from 40 N addition studies across four continents (fig. S1 and data S1). Through meta-analysis, we then investigated the role of enzyme activity and a wide range of environmental and experimental factors in determining changes in soil C storage with N addition.

RESULTS

Averaged across all studies, N addition significantly increased soil C storage by 11.0%. N addition significantly increased cellulase activity by 15.2% and repressed LME activity by 12.8% (Fig. 1A). Changes in soil C storage with N addition were negatively correlated with N suppression of LME activity, such that N-induced suppression of LME activity was associated with increases in soil C content (Fig. 1B). This negative relationship held over a range of ecosystems and N addition methods (figs. S2 and S3), although it was not significant for studies with high soil C/N ratios (>21.4; fig. S4). The response of LME activity explained 40.4% of the variation in soil C storage to N addition. In contrast, the effects of N addition on soil C storage were unrelated to the responses of cellulase activity (Fig. 1C). A model selection analysis (see Materials and Methods) confirmed that responses of soil C storage were best predicted by N-induced changes in LME activity over a broad range of climate factors, vegetation and soil types, and N application methods (Fig. 2). The response of LME activity also explained more variation in the response of soil C compared to a wide range of additional factors considered in the analysis (table S2; these factors were reported for only subsets of studies and so were analyzed individually).

Across the data set, N addition significantly decreased soil pH by 0.10 U (95% confidence interval, 0.02 to 0.17). Low soil pH can reduce decomposition rates and promote soil C storage (*21*, *23*, *24*). Thus, for the subset of studies reporting soil pH, we repeated our model selection procedure, including soil pH and treatment effects on soil pH as predictors. Responses of LME activity remained the most essential predictor of the effects of N addition on soil C storage (fig. S5). In addition, N addition also significantly increased the soil recalcitrant C pool by 22.7% and the proportion of recalcitrant C to total soil C storage by 9.2% (Fig. 3).

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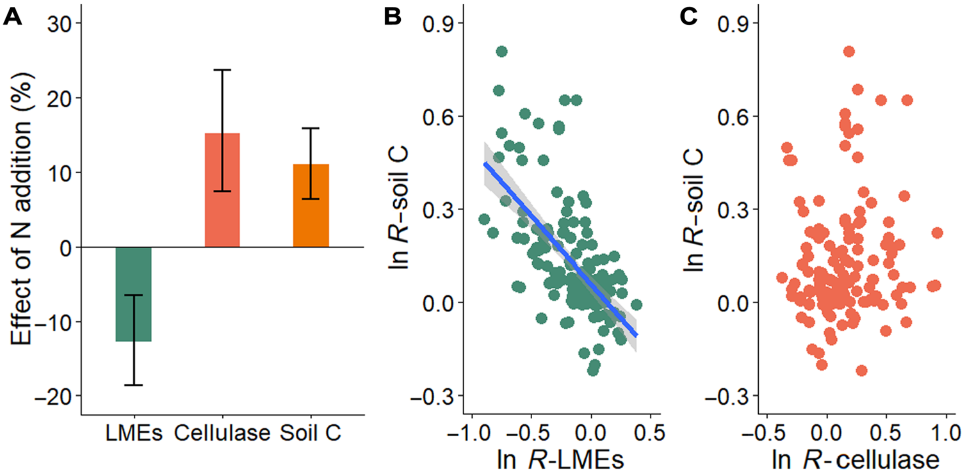

Fig. 1. Effects of N addition on LME activity, cellulase activity, and soil C storage (**A**). Relationship between the responses (ln *R*) of soil C storage to N addition and the response of LME activity (**B**) and cellulase activity (**C**). Error bars represent 95% confidence intervals; $n = 146$ in each panel. A negative relationship was found between the response of LME activity and the response of soil C storage [coefficient of determination (r^2) = 0.404, $P < 0.001$]. The light gray area indicates the confidence interval around the regression line. No significant relationship was found between the response of cellulase activity and the response of soil C storage ($r^2 = 0.008$, $P = 0.295$).

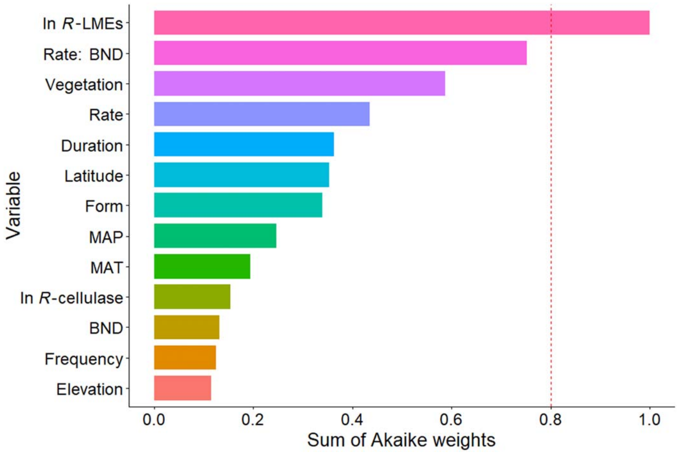

Fig. 2. Model-averaged importance of the predictors of the effects of N addition on soil C storage. The importance value is based on the sum of Akaike weights derived from model selection using corrected Akaike's information criteria. Cutoff is set at 0.8 to differentiate between essential and nonessential predictors. In *R*, log-transformed response ratio; BND, background N deposition; MAP, mean annual precipitation; MAT, mean annual temperature; duration, rate, frequency, and form refer to difference in N addition methods (see Materials and Methods).

DISCUSSION

Why do cellulase activity and LME activity respond differently to N addition? First, microbes are more likely to produce LMEs when they are suffering from N limitation because N-containing molecules are often physically and chemically shielded by recalcitrant substrates such as lignin (*25*, *26*). Thus, by alleviating microbial N limitation and reinforcing microbial C limitation (*20*, *27*), N additions may stimulate cellulase activity and suppress LME activity (*18*, *25*). This explanation is consistent with our finding that the relationship between LME activity and soil C storage is absent in ecosystems with high soil C/N ratios; under these conditions, N additions are less likely to alleviate N limitation (*14*). Second, the difference in response could be related to changes in microbial community structure. Cellulase is produced by a large number of microorganisms, but only a small number of microorganisms secrete LMEs (for example, white-rot basidiomycetes and xylacarious ascomycetes) (*14*). N addition often reduces the abundance of microorganisms that secrete LMEs (*28*, *29*), although the mechanism underlying this response is still unclear. Third, N addition may also affect enzyme activity through its effect on soil pH. Soil pH can affect microbial physiology, binding of substrates to enzymes, and the formation of the enzyme protein (*21*). Because the optimal pH for cellulase activity is much lower than the optimal pH for LME activity (*20*), N-induced decreases in soil pH (*23*) may contribute to repressed LME activity (*6*). However, treatment effects on soil pH were small and could not predict soil C storage with N addition within this data set.

LMEs are predominantly associated with the decomposition of chemically recalcitrant substrates (*30*). Thus, our finding of N-induced increases in recalcitrant soil C is consistent with our interpretation that N addition stimulates soil C accumulation by reducing LME activity (*31*, *32*); it also corroborates a recent comprehensive meta-analysis on N-induced changes in recalcitrant soil C (*33*). Because these recalcitrant substrates protect the degradation of more labile material (*30*) and the degradation of these substrates constitute the rate-limiting step in soil organic matter (SOM) decomposition (*34*, *35*), our results strongly suggest that reduced microbial decomposition is a key process contributing to soil C sequestration with N addition (*2*). Our findings could also help to improve the predictive power of land C cycle models. Current model formulations of soil C dynamics are based on C input regulated by plant productivity and on SOM decomposition modulated by the Arrhenius equation (*30*); thus, these models lack the critical process of enzyme-mediated decomposition (*30*, *36*). However, a new generation of models that explicitly represent microbial activity may result in more accurate soil C predictions (*37*). Our results further highlight the necessity of taking the microbial enzyme–mediated decomposition process into consideration to improve model predictions of soil C dynamics under global environmental change.

Our study shows that N-induced suppression of LME activity exerts more control over soil C storage than a broad suite of climatic and edaphic factors, and this control occurs across experimental N application methods and ecosystem types. The negative response of

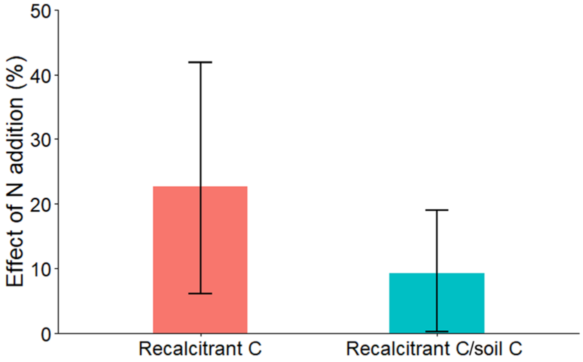

Fig. 3. Effects of N addition on the soil recalcitrant C pool. Error bars represent 95% confidence intervals ($n = 31$).

LME activity to N addition appears to override effects of N addition on various processes that could promote soil C loss, such as N-induced changes in substrate quality, microbial biomass, and priming through enhanced C input (*4*, *11*, *34*). Future research needs to identify the microbial and molecular mechanisms underlying the suppression of LME activity and their controlling factors. The strong role of LMEs in modulating changes in soil C storage suggests that understanding this enzyme system will reveal an independent and microbially mediated control of soil C sink in terrestrial ecosystems.

MATERIALS AND METHODS

Data collection

We used Web of Science (http://apps.webofknowledge.com/), Google Scholar (http://scholar.google.com/), and China National Knowledge Infrastructure (www.cnki.net/) for an exhaustive search of articles published before March 2018. The keywords and phrases used for literature research were as follows: (i) "nitrogen addition," "nitrogen amendment," "nitrogen enrichment," "nitrogen fertilizer," "nitrogen elevated," or "nitrogen deposition"; (ii) "glucosidase," "cellobiosidase," "xylosidase," "peroxidase," "phenol oxidase," "polyphenol oxidase," "lignin modifying enzymes," or "cellulase"; (iii) "soil carbon"; and (iv) "terrestrial," "soil," or "land".

To be included in our data set, articles had to meet several requirements. First, we only considered experiments that lasted at least 1 year. Second, control and N addition treatments had to be applied at the same experimental site; that is, the microclimate, vegetation, and soil types were similar between treatments. Third, SDs and replicates had to be reported or could be derived from the results. Fourth, details on N addition methods (rate, frequency, form, and duration) had to be provided. We identified 40 studies that met these criteria, and 9 of these studies reported soil C data from the matching studies (see Supplementary Materials and Methods and data S1).

For each study, we recorded LME activity and cellulase activity (see Supplementary Materials and Methods and table S1), site location (longitude and latitude) and climatic variables (MAP and MAT), elevation, BND, vegetation and soil types, and N addition methods (rate, duration, frequency, and form of N addition). 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/), BND from the Global N deposition database (http://webmap.ornl.gov/). We classified vegetation types according to the Whittaker Biome Diagram (*38*), and soil types according to the Food and Agriculture Organization taxonomy (www.fao.org/soils-portal/soil-survey/soil-classification/usda-soil-taxonomy/en). Where available, we also tabulated plant productivity, soil pH, soil C/N, microbial abundance, soil texture, and the size of the recalcitrant C pool (see Supplementary Materials and Methods and data S2 and S3). When results were presented graphically, we used Engauge Digitizer 4.1 (http://digitizer.sourceforge.net) to digitize the data.

Data analysis

We evaluated the effects of N additions by the natural log of the response ratio (ln *R*), a metric commonly used in meta-analysis (*20*, *39*, *40*)

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

with X_C and X_N as the arithmetic mean values of the variables in the ambient and N addition treatments, respectively. The variances (v) of ln *R* are calculated by

$$v = \frac{S_N^2}{n_N X_N^2} + \frac{S_C^2}{n_C X_C^2} \quad (2)$$

with n_C and n_N as the replicate numbers and S_C and S_N as the SDs for ambient and N addition treatments, respectively.

Meta-analysis was conducted using the "rma.mv" function in the R package "metafor" (http://cran.r-project.org/web/packages/metafor/index.html). Because several papers contributed more than one response ratio, we included the variable "publication" as a random factor (*39*, *40*). The effects of N addition were considered significant if the 95% confidence interval did not overlap with zero. The results were reported as percentage change with N addition [that is, $100 \times (e^{\ln R} - 1)$] to ease interpretation.

The meta-analytic models were selected by using the same approach as in van Groenigen *et al.* (*39*) and Terrer *et al.* (*40*). Briefly, we analyzed all possible combinations of the studied factors in a mixed-effects meta-regression model using the "glmulti" package in R (www.metafor-project.org/doku.php/tips:model_selection_with_glmulti). The importance of each predictor was expressed as the sum of Akaike weights for models that included this factor, which can be considered as the overall support for each variable across all models. A cutoff of 0.8 was set to differentiate between essential and nonessential predictors. We evaluated the impacts of soil pH, soil C/N, soil texture (clay content), and N-induced changes in plant productivity, soil pH, soil C/N, and microbial community on soil C storage using linear regression analysis in R.

SUPPLEMENTARY MATERIALS

Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/4/8/eaaq1689/DC1

Supplementary Materials and Methods

Fig. S1. Global distribution of N addition experiments included in this meta-analysis.

Fig. S2. Relationships between the responses (ln *R*) of soil C storage and LME activity to N addition for various vegetation and soil types.

Fig. S3. Relationships between the responses (ln *R*) of soil C storage and LME activity to N addition for various N addition methods.

Fig. S4. Relationships between the responses (ln *R*) of soil C storage and LME activity to N addition for studies categorized by soil C/N ratio.

Fig. S5. Model-averaged importance of the predictors of the effects of N addition on soil C storage for studies that simultaneously reported soil pH in ambient and N addition treatments.

Fig. S6. Effects of N addition on cellulase activity and LME activity for all studies in our data set (that is, data S5).
Table S1. A detailed overview of the enzymes included in our meta-analysis.
Table S2. Evaluation of the model parameters used to explain soil C storage under N addition.
Data S1. Database of N addition studies reporting soil C storage and cellulase activity and LME activity that were used in our analysis.
Data S2. Database of N addition studies reporting plant productivity, soil C storage, soil pH, soil C/N, microbial community, and soil texture that were used in our analysis.
Data S3. Database of N addition studies reporting soil recalcitrant C pool that were used in our analysis.
Data S4. Database of N addition studies reporting cellulase activity and LME activity, but not soil C storage.
Data S5. Database of N addition studies reporting individual components of cellulase activity and LME activity.
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