A keystone microbial enzyme for nitrogen control of soil carbon storage

Ji Chen1,2,3, Yiqi Luo4,5*, Kees Jan van Groenigen6, Bruce A. Hungate3, Junji Cao2,7, Xuhui Zhou8,9, Rui-wu Wang1

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 active 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 extra-cellular 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. S1a 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).

1Center for Ecological and Environmental Sciences, Key Laboratory for Space Bioscience and Biotechnology, Northwestern Polytechnical University, Xi’an 710072, China. 2State Key Laboratory of Loess and Quaternary Geology (SKLLQG) and Key Laboratory of Aerosol Chemistry and Physics, Institute of Earth Environment, Chinese Academy of Sciences, Xi’an 710061, China. 3Aarhus University Centre for Circular Bioeconomy, Department of Agroecology, Aarhus University, Blichers Allé 20, 8830 Tjele, Denmark. 4Department for Earth System Science, Tsinghua University, Beijing 100084, China. 5Center for Ecosystem Science and Society and Department of Biological Sciences, Northern Arizona University, AZ 86011, USA. 6Department of Geography, College of Life and Environmental Sciences, University of Exeter, Exeter EX4 4QG, UK. 7Institute of Global Environmental Change, Xi’an Jiaotong University, Xi’an 710049, China. 8Center for Global Change and Ecological Forecasting, Tian Tong National Field Observation Station for Forest Ecosystem, School of Ecological and Environmental Sciences, East China Normal University, Shanghai 200062, China. 9Shanghai Institute of Pollution Control and Ecological Security, Shanghai 200437, China.

*Corresponding author. Email: luoyiqi@tsinghua.edu.cn
**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 xylaraceous 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...
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 microbiologically 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,” “xylanase,” “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)

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

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

$$\nu = \frac{S_N^2}{n_X X_N^2} + \frac{S_C^2}{n_E X_C^2}$$

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 “lme4” 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 × ($e^{\ln R} - 1$)] 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 “gmult” package in R (www.metafor-project.org/doku.php/tipsmodel_selection_with_gmult). The importance of each predictor was expressed as the sum of Akaikes 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.

Chen et al., Sci. Adv. 2018;4:eaaq1689 22 August 2018
REFERENCES AND NOTES

1. J. N. Galloway, A. R. Townsend, J. W. Erisman, M. Bekunda, Z. Cai, J. R. Freneny, L. A. Martellini, S. P. Seitzinger, M. A. Sutton, Transformation of the nitrogen cycle: Recent trends, questions, and potential solutions. Science 320, 889–892 (2008).

2. D. S. Reay, F. Dentener, P. Smith, J. Grace, R. A. Feely, Global nitrogen deposition and carbon sinks. Nat. Geosci. 1, 430–437 (2008).

3. A. B. Hungate, J. S. Dukes, M. R. Shayer, Y. Luo, C. B. Field, Nitrogen and climate change. Science 302, 1512–1513 (2003).

4. I. A. Janssens, W. Dieleman, S. Luyssaert, J.-A. Subke, M. Reichstein, R. Ceulemans, P. Ciais, A. J. Dolman, J. Grace, G. Matteucci, D. Papale, S. L. Piao, E.-D. Schulze, J. Tang, B. E. Law, Reduction of forest soil respiration in response to nitrogen deposition. Nat. Geosci. 3, 315–322 (2010).

5. L. Liu, T. L. Greaver, A global perspective on belowground carbon dynamics under nitrogen enrichment. Ecol. Lett. 13, 819–828 (2010).

6. M. Lu, X. Zhou, Y. Luo, Y. Yang, C. Fang, J. Chen, B. Li, Minor stimulation of soil carbon storage by nitrogen addition: A meta-analysis. Agr. Ecosyst. Environ. 140, 234–244 (2011).

7. L. E. Nave, E. D. Vance, C. W. Swanston, P. S. Curtis, Impacts of elevated N inputs on north temperate forest soil C storage, C/N, and net N mineralization. Geoderma 153, 231–240 (2009).

8. M. C. Mack, E. A. G. Schuur, M. S. Breet-Harte, G. R. Shaver, F. S. Chapin, Ecosystem carbon storage in arctic tundra reduced by long-term nutrient fertilization. Nature 431, 440–443 (2004).

9. M. Knorr, S. D. Frey, P. S. Curtis, Nitrogen additions and litter decomposition: A meta-analysis. Ecology 86, 3252–3257 (2005).

10. C. Verell, B. Waring, Nitrogen limitation of decomposition and decay: How can it occur? Ecol. Chem. Phys. 24, 1417–1427 (2018).

11. J. C. Neff, A. R. Townsend, G. Gleinzer, S. J. Lehman, J. Turnbull, W. D. Bowman, Variable effects of nitrogen additions on the stability and turnover of soil carbon. Nature 419, 915–917 (2002).

12. S. D. Allison, T. B. Gartner, M. C. Mack, K. McGuire, K. Treseder, Nitrogen alters carbon dynamics during early succession in boreal forest. Soil Biol. Biochem. 42, 1157–1164 (2010).

13. R. Hyvönen, T. Persson, S. Andersson, B. Olsson, G. I. Ägren, S. Linder, Impact of long-term nitrogen addition on carbon stocks in trees and soils in northern Europe. Biogeochemistry 89, 121–137 (2008).

14. M. M. Carreño, R. L. Sinsabaugh, D. A. Repert, D. F. Parkhurst, Microbial enzyme shifts explain litter decay responses to simulated nitrogen deposition. Ecology 81, 2359–2365 (2000).

15. F. Rinaeu, D. Roth, F. Shah, M. Stams, T. Johansson, B. Canbäck, P. B. Olsen, P. Persson, M. N. Geil, E. Lindqvist, I. V. Geoglovié, L. Lange, A. Tunlid, The ectomycorrhizal fungus Paxillus involutus converts organic matter in plant litter using a trimmed brown-rot mechanism involving Fenton chemistry. Environ. Microbiol. 14, 1477–1487 (2012).

16. F. Shah, C. Nicolas, J. Benitez, M. Elshtröm, M. Smits, F. Rinaeu, B. Canbäck, D. Floudas, R. Carleer, G. Lackner, J. Braesel, D. Hoffmeister, B. Henrissat, A. Tunlid, Ectomycorrhizal fungal decompose soil organic matter using oxidative mechanisms adapted from saprotrophic ancestors. New Phytol. 209, 1705–1719 (2016).

17. B. L. Keeler, S. E. Hobbie, L. E. Kellogg, Effects of long-term nitrogen addition on microbial enzyme activity in eight forested and grassland sites: Implications for litter and soil organic matter decomposition. Ecosystems 12, 1–15 (2009).

18. R. G. Burns, J. L. DeForest, J. Marxsen, R. L. Sinsabaugh, M. E. Stromberger, M. D. Wallenstein, M. N. Weintraub, A. Zoppini, Soil enzymes in a changing environment: Current knowledge and future directions. Soil Biol. Biochem. 58, 216–234 (2013).

19. S. Jian, L. Ji, J. Chen, G. Wang, M. A. Mayers, K. E. Dzantor, D. Hui, Y. Luo, Soil extracellular enzyme activities, soil carbon and nitrogen storage under nitrogen fertilization: A meta-analysis. Soil Biol. Biochem. 101, 32–43 (2016).
J. L. DeForest, D. R. Zak, K. S. Pregitzer, A. J. Burton, Atmospheric nitrate deposition and its effects on grassland soil carbon cycling but not storage. *Ecosystems* 14, 234–247 (2011).

Q. Wang, P. Tian, S. Liu, T. Sun, Inhibition effects of N deposition on soil organic carbon storage. *Soil Biol. Biochem.* 42, 2161–2173 (2010).

M. G. Lovett, M. A. Arthur, K. C. Weathers, Effects of tree species and N additions on forest floor microbial communities and extracellular enzyme activities. *Soil Biol. Biochem.* 42, 347–360 (2010).

Y. Zhang, C. Wang, K. Xu, X. Yang, Effect of simulated nitrogen deposition on soil enzyme activities and microbial community functional diversities in a Chinese Fir plantation. *Soils* 45, 120–128 (2013).

L. H. Yu, W. Ding, J. Luo, R. Geng, A. Ghani, Z. Cai, Effects of long-term compost and fertilizer application on stability of aggregate-associated organic carbon in an intensively cultivated sandy loam soil. *Biol. Fertil. Soils* 48, 325–336 (2012).

H. Y. Yu, W. X. Ding, J. Luo, A. Donnison, J. B. Zhang, Long-term effect of compost and inorganic fertilizer on activities of carbon-cycle enzymes in aggregates of an intensively cultivated sandy loam. *Soil Use Manage.* 28, 347–360 (2012).

Y. Yuan, H. Fan, W. Liu, R. Huang, F. Shen, F. Hu, H. Li, Effects of simulated nitrogen deposition on soil enzyme activities and microbial community functional diversity in a Chinese Fir plantation. *Soils* 45, 120–128 (2013).

L. H. Zeglin, M. Stursova, R. L. Sinsabaugh, S. L. Collins, Microbial responses to nitrogen addition in three contrasting grassland ecosystems. *Oecologia* 154, 349–359 (2007).

Q. Zhang, G. Liang, W. Zhou, J. Sun, X. Wang, P. He, Fatty-acid profiles and enzyme activities in soil particle-size fractions under long-term fertilization. *Soil Sci. Soc. Am. J.* 69, 97–111 (2005).

Y. Zhang, C. Wang, K. Xu, X. Yang, Effect of simulated nitrogen deposition on soil enzyme activities in a temperate forest. *Acta Ecol. Sin.* 37, 39–48 (2007).

S. A. Grandy, D. S. Salam, C. W. Wilson, J. P. Reynolds, D. W. Culp, S. S. Snapp, Soil respiration and litter decomposition responses to nitrogen fertilization rate in no-till corn systems. *Agric. Ecosyst. Environ.* 179, 35–40 (2013).

J. Gao, E. Wang, W. Ren, X. Liu, Y. Chen, Y. Shi, Y. Yang, Effects of simulated climate change on soil microbial biomass and enzyme activities in young Chinese fir (*Cunninghamia lanceolata*) in subtropical China. *Acta Ecol. Sin.* 37, 272–278 (2017).

A. S. Grandy, R. L. Sinsabaugh, J. C. Neff, M. Stursova, D. R. Zak, Nitrogen deposition effects on soil organic matter chemistry are linked to variation in enzymes, ecosystems and size fractions. *Biogeochemistry* 91, 37–49 (2008).

A. S. Grandy, D. S. Salam, J. Adams, C. W. Wilson, J. P. Reynolds, S. W. Culp, S. S. Snapp, Soil respiration and litter decomposition responses to nitrogen fertilization rate in no-till corn systems. *Agric. Ecosyst. Environ.* 179, 35–40 (2013).

S. E. Hobbie, W. C. Eddy, C. R. Buyarski, E. C. Adair, M. L. Ogdahl, P. Weisenhorn, Response of decomposing litter and its microbial community to multiple forms of nitrogen enrichment. *Ecol. Monogr.* 82, 389–405 (2012).

D. A. Fornara, D. Tilman, Soil carbon sequestration in prairie grasslands increased by chronic nitrogen addition. *Ecology* 93, 2030–2036 (2012).

J. X. Jiang, X. Y. Feng, H. Zhou, B. Z. Xu, J. S. He, Neutral effect of nitrogen addition and negative effect of phosphorus addition on topsoil extracellular enzymatic activities in an alpine grassland ecosystem. *Appl. Soil Ecol.* 107, 205–213 (2016).

D. He, X. Xiang, J.-S. He, C. Wang, G. Cao, J. Adams, H. Chu, Composition of the soil fungal community is more sensitive to phosphorus than nitrogen addition in the alpine meadow on the Qinghai-Tibetan Plateau. *Biol. Fertil. Soils* 52, 1059–1072 (2016).

J. Y. Jung, R. Lai, D. A. N. Ussiri, Changes in CO₂, NO₃⁻ abundance, inorganic nitrogen, β-glucosidase, and oxidative enzyme activities of soil during the decomposition of switchgrass root carbon as affected by inorganic nitrogen additions. *Biol. Fertil. Soils* 47, 801–813 (2011).

H. Kim, H. Kang, The impacts of excessive nitrogen additions on enzyme activities and nutrient leaching in two contrasting forest soils. *J. Microbiol.* 49, 369–375 (2011).

Y.-p Li, T.-x He, Q.-k Wang, Impact of fertilization on soil organic carbon and enzyme activities in a *Cunninghamia lanceolata* plantation. *Chin. J. Ecol.* 35, 1–10 (2016).

X. Liu, J. Wang, X. Zhao, Effects of simulated nitrogen deposition on soil enzyme activities in a Pinus tabulaeformis forest at the Taiyue Mountain. *Acta Ecol. Sin.* 35, 4613–4624 (2015).

L. Luo, H. Meng, R.-n. Wu, J.-D. Gu, Impact of nitrogen pollution/deposition on extracellular enzyme activity, microbial abundance and carbon storage in coastal mangrove sediment. *Chemosphere* 177, 275–283 (2017).

K. Lyverperumal, W. Shi, Soil enzyme activities in two foragin systems following application of different rates of swine lagoon effluent or ammonium nitrate. *Appl. Soil Ecol.* 38, 128–136 (2008).

N. S. Nowinska, S. E. Trombore, G. Jimenez, M. E. Fenn, Alteration of belowground carbon dynamics by nitrogen addition in southern California mixed conifer forests. *J. Geophys. Res.* 114, G02005 (2009).

A. J. Pinsonneault, T. R. Moore, N. T. Roulet, Effects of long-term fertilization on peat stoichiometry and associated microbial enzyme activity in an ombrotrophic bog. *Biogeochemistry* 129, 149–164 (2016).
98. D. R. Nemergut, A. R. Townsend, S. R. Sattin, K. R. Freeman, N. Fierer, J. C. Neff, W. D. Bowman, C. W. Schadt, M. N. Weintraub, S. K. Schmidt. The effects of chronic nitrogen fertilization on alpine tundra soil microbial communities: Implications for carbon and nitrogen cycling. *Environ. Microbiol.* **10**, 3093–3105 (2008).

99. X. Ren, J. Tang, J. Liu, H. He, D. Dong, Y. Cheng. Effects of elevated CO2 and temperature on soil enzymes of seedlings under different nitrogen concentrations. *J. Beijing For. Univ.* **36**, 44–53 (2014).

100. R. L. Sinsabaugh, M. M. Carreiro, D. A. Repert. Allocation of extracellular enzymatic activity in relation to litter composition, N deposition, and mass loss. *Biogeochemistry* **60**, 1–24 (2002).

101. S. Stark, M. K. Männistö, A. Eskelinen. Nutrient availability and pH jointly constrain microbial extracellular enzyme activities in nutrient-poor tundra soils. *Plant Soil* **383**, 373–385 (2014).

102. T. Sun, L. Dong, Z. Wang, X. Lü, Z. Mao. Effects of long-term nitrogen deposition on fine root decomposition and its extracellular enzyme activities in temperate forests. *Soil Biol. Biochem.* **93**, 50–59 (2016).

103. T. Sun, L. Dong, Z. Mao. Simulated atmospheric nitrogen deposition alters decomposition of ephemeral roots. *Ecosystems* **18**, 1240–1252 (2015).

104. K. N. Suding, I. W. Ashton, H. Bechtold, W. D. Bowman, M. L. Mobley, R. Winklemann. Plant and microbe contribution to community resilience in a directionally changing environment. *Ecol. Monogr.* **78**, 313–329 (2008).

105. Y. T. Zhao, X. F. Li, S. J. Han, Y. L. Hu. Soil enzyme activity under two forest types as affected by different levels of nitrogen deposition. *J. Appl. Ecol.* **19**, 2769–2773 (2008).

106. K. Yang, J. J. Zhu, S. Xu. Influences of various forms of nitrogen additions on carbon mineralization in natural secondary forests and adjacent larch plantations in Northeast China. *Can. J. For. Res.* **44**, 441–448 (2014).

107. D. Xuan, S. Song, Y. Yan, J. Weng, X. Song. The short-term responses of soil enzyme activities in Moso bamboo forest to simulated nitrogen deposition. *Ecol. Sci.* **33**, 1122–1128 (2014).

108. C. Wang, X. Feng, P. Guo, G. Han, X. Tian. Response of degradative enzymes to N fertilization during litter decomposition in a subtropical forest through a microcosm experiment. *Ecol. Res.* **25**, 1121–1128 (2010).

Acknowledgments

Funding: This study was supported by the Fundamental Research Funds for the Central Universities (3102016QD078), the National Natural Science Foundation of China (NSFC) (41701292), China Postdoctoral Science Foundation (2017M610647 and 2018T111091), the Natural Science Basic Research Plan in Shaanxi Province (2017JQ3041), the State Key Laboratory of Loess and Quaternary Geology (SKLLQG1602), the Key Laboratory of Aerosol Chemistry and Physics (KLACP-17-02), and the Institute of Earth Environment, Chinese Academy of Sciences. Contributions from Y.L.’s Ecolab to this study were financially supported by the U.S. Department of Energy (grant DE-SC00114085) and NSFC (grants EF 11137293 and OIA-1301789). This work was also supported by NSFC-Yunnan United fund (U1302267) and the National Science Fund for Distinguished Young Scholars (31325005). The authors also acknowledge the financial support from the China Scholarship Council. Author contributions: J. Chen, Y.L., K.J.v.G., and B.A.H. designed the study. J. Chen, X.Z., J. Cao, and R.-w.W. collected the data. J. Chen, Y.L., K.J.v.G., B.A.H., X.Z., J. Cao, and R.-w.W. collaborated on data synthesis and interpretation. J. Chen, Y.L., K.J.v.G., and B.A.H. wrote the manuscript. All authors contributed to revisions. Competing interests: The authors declare that they have no competing interests. Data and materials availability: All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials and figshare (https://figshare.com/s/37aa98b76a7ef51da2e2). Correspondence should be addressed to Y.L. (luoyiqi@tsinghua.edu.cn). Requests for additional materials and database should be addressed to J. Cao (cao@loess.igex.ac.cn), R.-w.W. (wangrw@nwpu.edu.cn), and X.Z. (xzhou@des.ecnu.edu.cn).

Submitted 27 October 2017
Accepted 15 July 2018

101126/sciadv.aaq1689

Citation: J. Chen, Y. Luo, K. J. van Groenigen, B. A. Hungate, J. Cao, X. Zhou, R.-w. Wang. A keystone microbial enzyme for nitrogen control of soil carbon storage. *Sci. Adv.* **4**, eaaq1689 (2018).
A keystone microbial enzyme for nitrogen control of soil carbon storage
Ji Chen, Yiqi Luo, Kees Jan van Groenigen, Bruce A. Hungate, Junji Cao, Xuhui Zhou and Rui-wu Wang

Sci Adv 4 (8), eaaq1689.
DOI: 10.1126/sciadv.aaq1689