

ECOLOGY

A keystone microbial enzyme for nitrogen control of soil carbon storage

Ji Chen^{1,2,3}, Yiqi Luo^{4,5*}, Kees Jan van Groenigen⁶, Bruce A. Hungate⁵, Junji Cao^{2,7}, Xuhui Zhou^{8,9}, 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 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 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. 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).

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, Tiantong 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

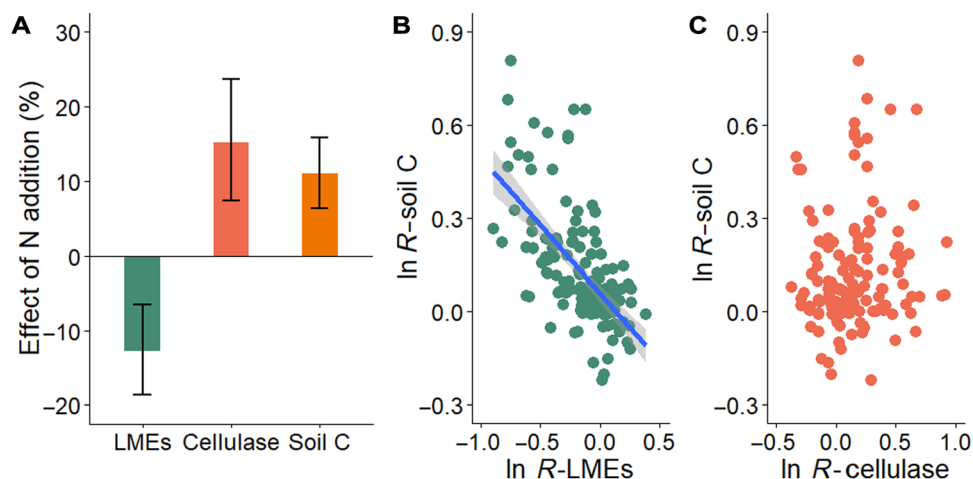


Fig. 1. Effects of N addition on LME activity, cellulase activity, and soil C storage (A). Relationship between the responses ($\ln R$) of soil C storage to N addition and the response of LME activity (B) and cellulase activity (C). Error bars represent 95% confidence intervals; $n = 146$ in each panel. 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 ($r^2 = 0.008$, $P = 0.295$).

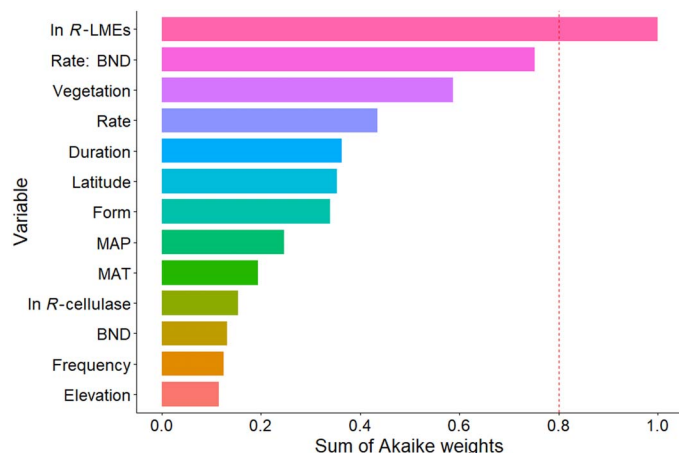


Fig. 2. Model-averaged importance of the predictors of the effects of N addition on soil C storage. The importance value is based on the sum of Akaike weights derived from model selection using corrected Akaike's information criteria. Cutoff is set at 0.8 to differentiate between essential and nonessential 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 mi-

croorganisms, but only a small number of microorganisms secrete LMEs (for example, white-rot basidiomycetes and xylicarious 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

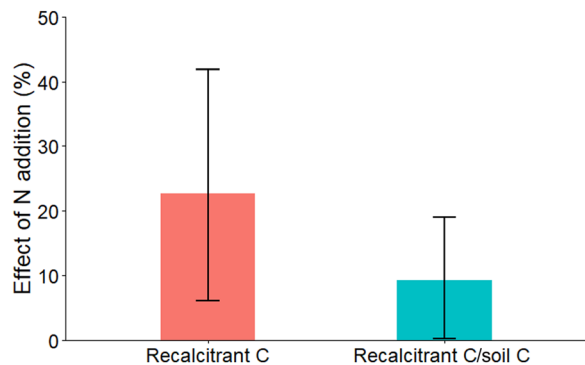


Fig. 3. Effects of N addition on the soil recalcitrant C pool. Error bars represent 95% confidence intervals ($n = 31$).

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/eaq1689/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.

References (41–108)

REFERENCES AND NOTES

- J. N. Galloway, A. R. Townsend, J. W. Erisman, M. Bekunda, Z. Cai, J. R. Freney, L. A. Martinelli, S. P. Seitzinger, M. A. Sutton, Transformation of the nitrogen cycle: Recent trends, questions, and potential solutions. *Science* **320**, 889–892 (2008).
- D. S. Reay, F. Dentener, P. Smith, J. Grace, R. A. Feely, Global nitrogen deposition and carbon sinks. *Nat. Geosci.* **1**, 430–437 (2008).
- B. A. Hungate, J. S. Dukes, M. R. Shaw, Y. Luo, C. B. Field, Nitrogen and climate change. *Science* **302**, 1512–1513 (2003).
- I. A. Janssens, W. Dieleman, S. Luysaert, 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).
- L. Liu, T. L. Greaver, A global perspective on belowground carbon dynamics under nitrogen enrichment. *Ecol. Lett.* **13**, 819–828 (2010).
- 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).
- 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).
- M. C. Mack, E. A. G. Schuur, M. S. Bret-Harte, G. R. Shaver, F. S. Chapin, Ecosystem carbon storage in arctic tundra reduced by long-term nutrient fertilization. *Nature* **431**, 440–443 (2004).
- M. Knorr, S. D. Frey, P. S. Curtis, Nitrogen additions and litter decomposition: A meta-analysis. *Ecology* **86**, 3252–3257 (2005).
- C. Averill, B. Waring, Nitrogen limitation of decomposition and decay: How can it occur? *Glob. Change Biol.* **24**, 1417–1427 (2018).
- J. C. Neff, A. R. Townsend, G. Gleixner, 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).
- 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).
- R. Hyvönen, T. Persson, S. Andersson, B. Olsson, G. I. Ågren, S. Linder, Impact of long-term nitrogen addition on carbon stocks in trees and soils in northern Europe. *Biogeochemistry* **89**, 121–137 (2008).
- M. M. Carreiro, 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).
- F. Rineau, D. Roth, F. Shah, M. Smits, T. Johansson, B. Canbäck, P. B. Olsen, P. Persson, M. N. Grell, E. Lindquist, I. V. Grigoriev, 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).
- F. Shah, C. Nicolás, J. Bentzer, M. Ellström, M. Smits, F. Rineau, B. Canbäck, D. Floudas, R. Carleer, G. Lackner, J. Braesel, D. Hoffmeister, B. Henrissat, D. Ahrén, T. Johansson, D. S. Hibbett, F. Martin, P. Persson, A. Tunlid, Ectomycorrhizal fungi decompose soil organic matter using oxidative mechanisms adapted from saprotrophic ancestors. *New Phytol.* **209**, 1705–1719 (2016).
- 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).
- 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).
- S. Jian, J. Li, J. Chen, G. Wang, M. A. Mayes, 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. Chen, Y. Luo, J. Li, X. Zhou, J. Cao, R.-W. Wang, Y. Wang, S. Shelton, Z. Jin, L. M. Walker, Z. Feng, S. Niu, W. Feng, S. Jian, L. Zhou, Costimulation of soil glycosidase activity and soil respiration by nitrogen addition. *Glob. Change Biol.* **23**, 1328–1337 (2017).
- R. L. Sinsabaugh, Phenol oxidase, peroxidase and organic matter dynamics of soil. *Soil Biol. Biochem.* **42**, 391–404 (2010).
- A. T. Austin, C. L. Ballaré, Dual role of lignin in plant litter decomposition in terrestrial ecosystems. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 4618–4622 (2010).
- D. Tian, S. Niu, A global analysis of soil acidification caused by nitrogen addition. *Environ. Res. Lett.* **10**, 024019 (2015).
- Z. Zhou, C. Wang, M. Zheng, L. Jiang, Y. Luo, Patterns and mechanisms of responses by soil microbial communities to nitrogen addition. *Soil Biol. Biochem.* **115**, 433–441 (2017).
- Y. Kuzyakov, X. Xu, Competition between roots and microorganisms for nitrogen: Mechanisms and ecological relevance. *New Phytol.* **198**, 656–669 (2013).
- S. Manzoni, R. B. Jackson, J. A. Trofymow, A. Porporato, The global stoichiometry of litter nitrogen mineralization. *Science* **321**, 684–686 (2008).
- R. ten Have, P. J. M. Teunissen, Oxidative mechanisms involved in lignin degradation by white-rot fungi. *Chem. Rev.* **101**, 3397–3414 (2001).
- K. Fog, The effect of added nitrogen on the rate of decomposition of organic matter. *Biol. Rev.* **63**, 433–462 (1988).
- T. Higuchi, Lignin biochemistry: Biosynthesis and biodegradation. *Wood Sci. Technol.* **24**, 23–63 (1990).
- E. A. Davidson, I. A. Janssens, Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature* **440**, 165–173 (2006).
- S. D. Frey, S. Ollinger, K. Nadelhoffer, R. Bowden, E. Brzostek, A. Burton, B. A. Caldwell, S. Crow, C. L. Goodale, A. S. Grandy, A. Finzi, M. G. Kramer, K. Lajtha, J. LeMoine, M. Martin, W. H. McDowell, R. Minocha, J. J. Sadowsky, P. H. Templer, K. Wickings, Chronic nitrogen additions suppress decomposition and sequester soil carbon in temperate forests. *Biogeochemistry* **121**, 305–316 (2014).
- Z. L. Rinkes, I. Bertrand, B. A. Z. Amin, A. S. Grandy, K. Wickings, M. N. Weintraub, Nitrogen alters microbial enzyme dynamics but not lignin chemistry during maize decomposition. *Biogeochemistry* **128**, 171–186 (2016).
- J. Liu, N. Wu, H. Wang, J. Sun, B. Peng, P. Jiang, E. Bai, Nitrogen addition affects chemical compositions of plant tissues, litter and soil organic matter. *Ecology* **97**, 1796–1806 (2016).
- S. Fontaine, S. Barot, P. Barré, N. Bdioui, B. Mary, C. Rumpel, Stability of organic carbon in deep soil layers controlled by fresh carbon supply. *Nature* **450**, 277–280 (2007).
- M. W. I. Schmidt, M. S. Torn, S. Abiven, T. Dittmar, G. Guggenberger, I. A. Janssens, M. Kleber, I. Kögel-Knabner, J. Lehmann, D. A. C. Manning, P. Nannipieri, D. P. Rasse, S. Weiner, S. E. Trumbore, Persistence of soil organic matter as an ecosystem property. *Nature* **478**, 49–56 (2011).
- Y. Luo, A. Ahlström, S. D. Allison, N. H. Batjes, V. Brovkin, N. Carvalhais, A. Chappell, P. Ciais, E. A. Davidson, A. Finzi, K. Georgiou, B. Guenet, O. Hararuk, J. W. Harden, Y. He, F. Hopkins, L. Jiang, C. Koven, R. B. Jackson, C. D. Jones, M. J. Lara, J. Liang, A. D. McGuire, W. Parton, C. Peng, J. T. Randerson, A. Salazar, C. A. Sierra, M. J. Smith, H. Tian, K. E. O. Todd-Brown, M. Torn, K. J. van Groenigen, Y. P. Wang, T. O. West, Y. Wei, W. R. Wieder, J. Xia, X. Xu, X. Xu, T. Zhou, Toward more realistic projections of soil carbon dynamics by Earth system models. *Glob. Biogeochem. Cycles* **30**, 40–56 (2016).
- W. R. Wieder, G. B. Bonan, S. D. Allison, Global soil carbon projections are improved by modelling microbial processes. *Nat. Clim. Chang.* **3**, 909–912 (2013).
- R. H. Whittaker, Classification of natural communities. *Bot. Rev.* **28**, 1–239 (1962).
- K. J. van Groenigen, C. W. Osenberg, C. Terrer, Y. Carrillo, F. A. Dijkstra, J. Heath, M. Nie, E. Pendall, R. P. Phillips, B. A. Hungate, Faster turnover of new soil carbon inputs under increased atmospheric CO₂. *Glob. Change Biol.* **23**, 4420–4429 (2017).
- C. Terrer, S. Vicca, B. A. Hungate, R. P. Phillips, I. C. Prentice, Mycorrhizal association as a primary control of the CO₂ fertilization effect. *Science* **353**, 72–74 (2016).
- A. Lorber, Error propagation and figures of merit for quantification by solving matrix equations. *Anal. Chem.* **58**, 1167–1172 (1986).
- J. Chen, Y. Luo, J. Xia, L. Jiang, X. Zhou, M. Lu, J. Liang, Z. Shi, S. Shelton, J. Cao, Stronger warming effects on microbial abundances in colder regions. *Sci. Rep.* **5**, 18032 (2015).
- E. D. Vance, P. C. Brookes, D. S. Jenkinson, An extraction method for measuring soil microbial biomass C. *Soil Biol. Biochem.* **19**, 703–707 (1987).
- A. Frostegård, E. Bååth, The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. *Biol. Fertil. Soils* **22**, 59–65 (1996).
- M. Gallo, R. Amonette, C. Lauber, R. L. Sinsabaugh, D. R. Zak, Microbial community structure and oxidative enzyme activity in nitrogen-amended north temperate forest soils. *Microb. Ecol.* **48**, 218–229 (2004).
- X. Jing, X. Chen, M. Tang, Z. Ding, L. Jiang, P. Li, S. Ma, D. Tian, L. Xu, J. Zhu, C. Ji, H. Shen, C. Zheng, J. Fang, B. Zhu, Nitrogen deposition has minor effect on soil extracellular enzyme activities in six Chinese forests. *Sci. Total Environ.* **607–608**, 806–815 (2017).
- M. P. Waldrop, D. R. Zak, R. L. Sinsabaugh, M. Gallo, C. Lauber, Nitrogen deposition modifies soil carbon storage through changes in microbial enzymatic activity. *Ecol. Appl.* **14**, 1172–1177 (2004).

48. D. Tian, L. Jiang, S. Ma, W. Fang, B. Schmid, L. Xu, J. Zhu, P. Li, G. Losapio, X. Jing, C. Zheng, H. Shen, X. Xu, B. Zhu, J. Fang, Effects of nitrogen deposition on soil microbial communities in temperate and subtropical forests in China. *Sci. Total Environ.* **607–608**, 1367–1375 (2017).
49. D. F. Cusack, M. S. Torn, W. H. McDowell, W. L. Silver, The response of heterotrophic activity and carbon cycling to nitrogen additions and warming in two tropical soils. *Glob. Change Biol.* **16**, 2555–2572 (2010).
50. S. D. Frey, M. Knorr, J. L. Parrent, R. T. Simpson, Chronic nitrogen enrichment affects the structure and function of the soil microbial community in temperate hardwood and pine forests. *For. Ecol. Manage.* **196**, 159–171 (2004).
51. S. Zhao, K. Li, W. Zhou, S. Qiu, S. Huang, P. He, Changes in soil microbial community, enzyme activities and organic matter fractions under long-term straw return in north-central China. *Agric. Ecosyst. Environ.* **216**, 82–88 (2016).
52. K. Yang, J. Zhu, J. Gu, S. Xu, L. Yu, Z. Wang, Effects of continuous nitrogen addition on microbial properties and soil organic matter in a *Larix gmelinii* plantation in China. *J. For. Res.* **29**, 85–92 (2018).
53. H. Chung, D. R. Zak, P. B. Reich, D. S. Ellsworth, Plant species richness, elevated CO₂, and atmospheric nitrogen deposition alter soil microbial community composition and function. *Glob. Change Biol.* **13**, 980–989 (2007).
54. J. P. Reid, E. C. Adair, S. E. Hobbie, P. B. Reich, Biodiversity, nitrogen deposition, and CO₂ affect grassland soil carbon cycling but not storage. *Ecosystems* **15**, 580–590 (2012).
55. J. L. DeForest, D. R. Zak, K. S. Pregitzer, A. J. Burton, Atmospheric nitrate deposition and the microbial degradation of cellobiose and vanillin in a northern hardwood forest. *Soil Biol. Biochem.* **36**, 965–971 (2004).
56. M. P. Waldrop, D. R. Zak, R. L. Sinsabaugh, Microbial community response to nitrogen deposition in northern forest ecosystems. *Soil Biol. Biochem.* **36**, 1443–1451 (2004).
57. Y. Du, P. Guo, J. Liu, C. Wang, N. Yang, S. Jiao, Different types of nitrogen deposition show variable effects on the soil carbon cycle process of temperate forests. *Glob. Change Biol.* **20**, 3222–3228 (2014).
58. 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).
59. 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).
60. A. S. Grandy, D. S. Salam, K. Wickings, M. D. McDaniel, S. W. Culman, 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).
61. 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).
62. D. A. Fornara, D. Tilman, Soil carbon sequestration in prairie grasslands increased by chronic nitrogen addition. *Ecology* **93**, 2030–2036 (2012).
63. X. Jing, X. Yang, F. Ren, H. Zhou, B. Zhu, 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).
64. 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).
65. J. Y. Jung, R. Lal, D. A. N. Ussiri, Changes in CO₂, ¹³C 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).
66. 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).
67. 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).
68. X. Liu, J. Wang, X. Zhao, Effects of simulated nitrogen deposition on the soil enzyme activities in a *Pinus tabulaeformis* forest at the Taiyue Mountain. *Acta Ecol. Sin.* **35**, 4613–4624 (2015).
69. 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).
70. K. Lyyemperumal, W. Shi, Soil enzyme activities in two forage systems following application of different rates of swine lagoon effluent or ammonium nitrate. *Appl. Soil Ecol.* **38**, 128–136 (2008).
71. N. S. Nowinski, S. E. Trumbore, 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).
72. 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).
73. M. O. Rappe-George, M. Choma, P. Čapek, G. Börjesson, E. Kaštovská, H. Šantrůčková, A. I. Gårdenäs, Indications that long-term nitrogen loading limits carbon resources for soil microbes. *Soil Biol. Biochem.* **115**, 310–321 (2017).
74. R. L. Sinsabaugh, M. E. Gallo, C. Lauber, M. P. Waldrop, D. R. Zak, Extracellular enzyme activities and soil organic matter dynamics for northern hardwood forests receiving simulated nitrogen deposition. *Biogeochemistry* **75**, 201–215 (2005).
75. M. Stursova, C. L. Crenshaw, R. L. Sinsabaugh, Microbial responses to long-term N deposition in a semiarid grassland. *Microb. Ecol.* **51**, 90–98 (2006).
76. X.-L. Sun, J. Zhao, Y.-M. You, O. J. Sun, Soil microbial responses to forest floor litter manipulation and nitrogen addition in a mixed-wood forest of northern China. *Sci. Rep.* **6**, 19536 (2016).
77. D. C. Thomas, D. R. Zak, T. R. Filley, Chronic N deposition does not apparently alter the biochemical composition of forest floor and soil organic matter. *Soil Biol. Biochem.* **54**, 7–13 (2012).
78. D. R. Zak, Z. B. Freedman, R. A. Upchurch, M. Steffens, I. Kögel-Knabner, Anthropogenic N deposition increases soil organic matter accumulation without altering its biochemical composition. *Glob. Change Biol.* **23**, 933–944 (2017).
79. L. K. Tiemann, S. A. Billings, Indirect effects of nitrogen amendments on organic substrate quality increase enzymatic activity driving decomposition in a Mesic grassland. *Ecosystems* **14**, 234–247 (2011).
80. Q. Wang, P. Tian, S. Liu, T. Sun, Inhibition effects of N deposition on soil organic carbon decomposition was mediated by N types and soil nematode in a temperate forest. *Appl. Soil Ecol.* **120**, 105–110 (2017).
81. M. P. Weand, M. A. Arthur, G. M. Lovett, R. L. McCulley, K. C. Weathers, Effects of tree species and N additions on forest floor microbial communities and extracellular enzyme activities. *Soil Biol. Biochem.* **42**, 2161–2173 (2010).
82. G. M. Lovett, M. A. Arthur, K. C. Weathers, R. D. Fitzhugh, P. H. Templer, Nitrogen addition increases carbon storage in soils, but not in trees, in an Eastern U.S. deciduous forest. *Ecosystems* **16**, 980–1001 (2013).
83. 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).
84. H. Y. Yu, W. X. Ding, J. F. 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).
85. 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 diversities in a Chinese Fir plantation. *Soils* **45**, 120–128 (2013).
86. 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).
87. 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.* **80**, 97–111 (2016).
88. 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**, (2017).
89. S. D. Allison, C. I. Czimczik, K. K. Treseder, Microbial activity and soil respiration under nitrogen addition in Alaskan boreal forest. *Glob. Change Biol.* **14**, 1156–1168 (2008).
90. C. Bai, M. Hong, G. Han, M. Zhao, B. Lu, Response of three kinds of enzyme activity to simulate warming and nitrogen addition. *J. Inn. Mong. Univ. (Natural Science Edition)* **43**, 509–614 (2012).
91. J. L. DeForest, D. R. Zak, K. S. Pregitzer, A. J. Burton, Atmospheric nitrate deposition and enhanced dissolved organic carbon leaching: Test of a potential mechanism. *Soil Sci. Soc. Am. J.* **69**, 1233–1237 (2005).
92. J. E. Drake, A. C. Oishi, M.-A. Giasson, R. Oren, K. H. Johnsen, A. C. Finzi, Trenching reduces soil heterotrophic activity in a loblolly pine (*Pinus taeda*) forest exposed to elevated atmospheric CO₂ and N fertilization. *Agric. For. Meteorol.* **165**, 43–52 (2012).
93. H. Fang, S. Cheng, E. Lin, G. Yu, S. Niu, Y. Wang, M. Xu, X. Dang, L. Li, L. Wang, Elevated atmospheric carbon dioxide concentration stimulates soil microbial activity and impacts water-extractable organic carbon in an agricultural soil. *Biogeochemistry* **122**, 253–267 (2015).
94. Z. B. Freedman, R. A. Upchurch, D. R. Zak, L. C. Cline, Anthropogenic N deposition slows decay by favoring bacterial metabolism: Insights from metagenomic analyses. *Front. Microbiol.* **7**, 259 (2016).
95. T. Kunito, Y. Akagi, H.-D. Park, H. Toda, Influences of nitrogen and phosphorus addition on polyphenol oxidase activity in a forested Andisol. *Eur. J. For. Res.* **128**, 361–366 (2009).
96. Y. Lü, C. Wang, Y. Jia, J. Du, X. Ma, W. Wang, G. Pu, X. Tian, Responses of soil microbial biomass and enzymatic activities to different forms of organic nitrogen deposition in the subtropical forests in East China. *Ecol. Res.* **28**, 447–457 (2013).
97. K. Min, H. Kang, D. Lee, Effects of ammonium and nitrate additions on carbon mineralization in wetland soils. *Soil Biol. Biochem.* **43**, 2461–2469 (2011).

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. Winkelman, 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 NSF (grants EF 1137293 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.llqg.ac.cn), R.-w.W. (wangrw@nwpu.edu.cn), and X.Z. (xhzhou@des.ecnu.edu.cn).

Submitted 27 October 2017

Accepted 15 July 2018

Published 22 August 2018

10.1126/sciadv.aqa1689

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

ARTICLE TOOLS

<http://advances.sciencemag.org/content/4/8/eaaq1689>

SUPPLEMENTARY MATERIALS

<http://advances.sciencemag.org/content/suppl/2018/08/20/4.8.eaaq1689.DC1>

REFERENCES

This article cites 107 articles, 5 of which you can access for free
<http://advances.sciencemag.org/content/4/8/eaaq1689#BIBL>

PERMISSIONS

<http://www.sciencemag.org/help/reprints-and-permissions>

Use of this article is subject to the [Terms of Service](#)

Science Advances (ISSN 2375-2548) is published by the American Association for the Advancement of Science, 1200 New York Avenue NW, Washington, DC 20005. 2017 © The Authors, some rights reserved; exclusive licensee American Association for the Advancement of Science. No claim to original U.S. Government Works. The title *Science Advances* is a registered trademark of AAAS.