of BCMA2 (Fig. 4) and visualized the locations of variations in the BCMA1 and BCMA3 proteins that might explain their altered catalytic functions. G134L, one of the two residues showing evidence for accelerated molecular evolution, occurs in substrate recognition site SRS1 near the heme (Fig. 4) and is predicted to alter the catalytic site space in the region closest to the heme. The other, P536K, occurs five amino acids upstream from their C termini and is predicted to alter electrostatic interactions of this flexible tail region. Mapping of the two positions varying between the BCMA3-MT and BCMA3-CO alleles indicates that $Val^{148} \rightarrow$ Leu occurs in a region potentially affecting interactions with electron transfer partners, and that $Met^{268} \rightarrow$ Val occurs in a SRS3 region predicted to affect the volume of the upper catalytic site and/or substrate access (fig. S6). However, determining the biochemical effects of these changes is beyond the scope of this study.

We have shown how the *BCMA* QTL affects plant chemistry and insect resistance, and thus fitness, in a quantitative manner. In *Boechera*, the *BCMA2* locus retains ancestral activity and synteny, whereas *BCMA1* and *BCMA3* have evolved novel catalytic activity. The resulting polymorphic Met-GS and BC-GS show heterogeneous effects on host plant resistance against diverse enemies across a range of environments. In the Montana population, homozygotes at *BCMA* produce BC-GS and show greater resistance to damage by a diverse community of herbivores (tables S4 and S6). Further evidence that these compounds have environment-dependent consequences comes from transgenic *Arabidopsis*, where BC-GS cause increased resistance to the pathogen *Erwinia carotovora* (*6*), and from other herbivores, where BC-GS cause increased susceptibility to *Trichoplusia ni* (*10*). However, *BCMA* has no effect on insect damage in Colorado (tables S4 and S6), where other loci control resistance (table S6). On the basis of this study, we conclude that heterogeneous responses to diverse biotic interactions in the context of selection by herbivores likely contribute to the genetic diversity of *BCMA*.

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Acknowledgments: We thank R. Colautti, K. Donohue, M. Feder, T. Pendergast, M. Rausher, C. Rushworth, A. Shumate, M. Wagner, J. Willis, and two anonymous reviewers for helpful comments. K. Springer, E. Ballweg, M. Cameron, K. Chu, S. Hurst, V. Cousins, K. Dales, R. Doll, J. Lutkenhaus, M. Mitchell-Olds, S. Mitchell-Olds, E. Raskin, J. Rivera, L. Saucier, M. Wagner, and T. Weiss-Lehman helped in lab and field. N. Wicks and Bitterroot National Forest allowed us to work on their property. We thank the Heald family for support and hospitality. Supported by NIH grants R01-GM086496 (T.M.-O.) and R01-GM079530 (M.A.S.), NSF grant EF-0723447 (T.M.-O.); NSF dissertation grants 1011167 (C.O.-M.) and 1110445 (C.-R.L.); and a Netherlands Organization for Scientific Research (NWO) Ecogenomics grant (M.E.S.). Data in the supplementary materials are also available as GenBank accession nos. JX185680, JX185681, and BCMA JQ337904 to BCMA JQ337909. Data are deposited in the Dryad Repository: http://dx.doi.org/10.5061/dryad.kc6m8.

Supplementary Materials

www.sciencemag.org/cgi/content/full/337/6098/1081/DC1
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8 March 2012; accepted 19 June 2012
10.1126/science.1221636

Arbuscular Mycorrhizal Fungi Increase Organic Carbon Decomposition Under Elevated CO_2

Lei Cheng,[1]* Fitzgerald L. Booker,[2,3] Cong Tu,[1] Kent O. Burkey,[2,3] Lishi Zhou,[1,4] H. David Shew,[1] Thomas W. Rufty,[3] Shuijin Hu[1]†

The extent to which terrestrial ecosystems can sequester carbon to mitigate climate change is a matter of debate. The stimulation of arbuscular mycorrhizal fungi (AMF) by elevated atmospheric carbon dioxide (CO_2) has been assumed to be a major mechanism facilitating soil carbon sequestration by increasing carbon inputs to soil and by protecting organic carbon from decomposition via aggregation. We present evidence from four independent microcosm and field experiments demonstrating that CO_2 enhancement of AMF results in considerable soil carbon losses. Our findings challenge the assumption that AMF protect against degradation of organic carbon in soil and raise questions about the current prediction of terrestrial ecosystem carbon balance under future climate-change scenarios.

Arbuscular mycorrhizal fungi (AMF), which form associations with roots of ~80% of land plant species, obtain carbon (C) from their host plants in return for mineral nutrients (*1*, *2*). AMF utilize a large proportion (up to 20%) of net plant photosynthates under ambient atmospheric CO_2 (aCO_2) (*3*, *4*), deposit slow cycling organic compounds such as chitin and glomalin (*1*, *5*), and protect organic matter from microbial attack by promoting soil aggregation (*6*). AMF thus play a critical role in the global C cycle. Atmospheric CO_2 enrichment often increases plant photosynthate allocation to AMF and stimulates the growth of AMF (*3*, *7*–*9*), leading to a proposition that global soils may sequester more C through mycorrhizal symbioses under future scenarios of elevated CO_2 (eCO_2) (*3*, *5*, *7*–*12*). This hypothesis, however, does not consider the effect of AMF on decomposition under eCO_2. Indeed, AMF growth can result in enhanced decomposition of complex organic material and alter plant N uptake (*13*–*15*).

We conducted four independent but complementary experiments to investigate how CO_2 stimulation of AMF affects organic C decomposition in soil and the subsequent N dynamics in the plant-soil system by combining dual $^{13}C/^{15}N$ labeling and hyphae-ingrowth techniques (*16*). We first ascertained the effect of eCO_2 [main plot, $n = 4$; ambient at 380 versus elevated at 580 parts per million by volume (ppmv)] and N addition (subplot; control at 0 versus added at 5 g N m^{-2}) on

[1]Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695, USA. [2]United States Department of Agriculture, Agricultural Research Service, Plant Science Research Unit, Raleigh, NC 27607, USA. [3]Department of Crop Science, North Carolina State University, Raleigh, NC 27695, USA. [4]State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, Chinese Academy of Sciences, Xiangshan, Beijing 100093, China.

*Present address: Department of Ecosystem Science and Management, The Pennsylvania State University, University Park, PA 16802, USA.

†To whom correspondence should be addressed. E-mail: shuijin_hu@ncsu.edu

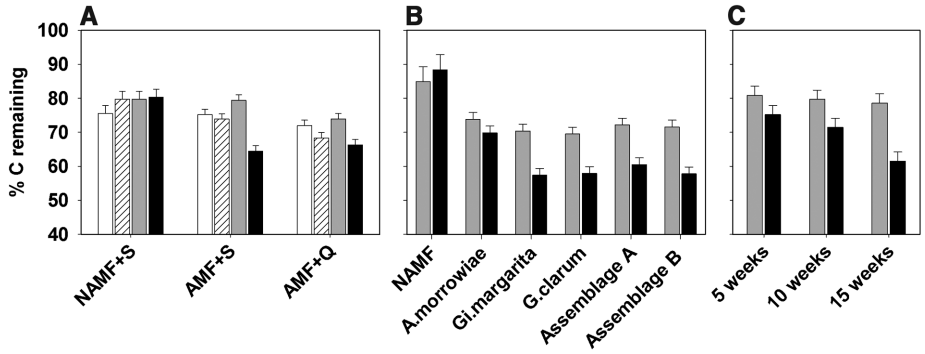

Fig. 1. The effect of arbuscular mycorrhizal fungi on organic C decomposition. (**A**) C remaining (%) within hyphae-ingrowth bags after 10 weeks of incubation under different CO_2 and N concentrations. +S and +Q refer to autoclaved sandy loam soil (S) and quartz sand (Q) in hyphae-ingrowth bags, respectively. Blank and gray bars denote ambient CO_2 without and with added N, respectively; hatched and black bars denote elevated CO_2 without and with added N, respectively. Data shown (means ± SEM) are based on the fitted mixed model. The main effects of N, and CO_2 × N and CO_2 × N × AMF interactions were not significant ($P > 0.05$). (**B** and **C**) C remaining (%) within hyphae-ingrowth cores after 10 weeks of incubation under different CO_2 and AMF species treatments (B) and within hyphae-ingrowth bags after 5, 10, and 15 weeks of incubation under different CO_2 concentrations in the field (C). Full AMF species name and assemblage composition are in table S1. Gray bars, ambient CO_2; black bars, elevated CO_2. Data shown (means ± SEM) are based on the fitted mixed model.

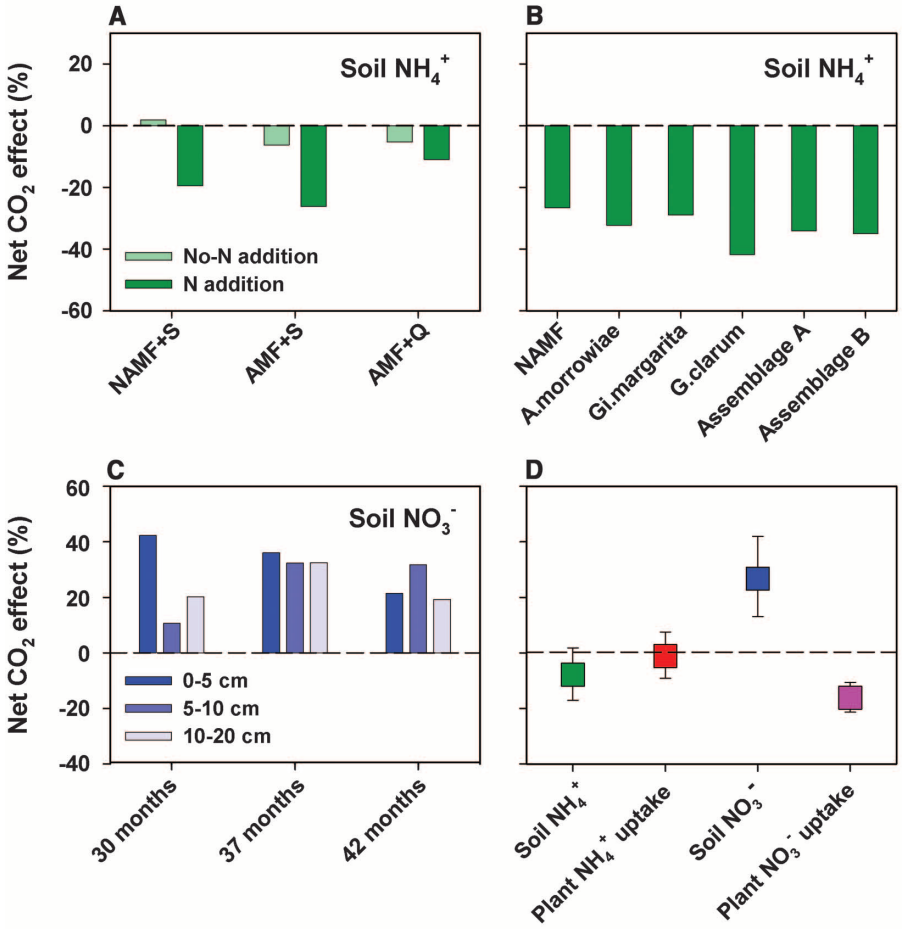

Fig. 2. Differential CO_2 effects on soil ammonium (NH_4^+) and nitrate (NO_3^-) and on plant NH_4^+ and NO_3^- uptake. (**A** to **C**) Net CO_2 effect (%) on soil NH_4^+ under different AMF and N concentrations (A) and different AMF species and assemblages (B) in microcosms, and on soil NO_3^- of three soil layers in the field (C). (**D**) A meta-analysis of net CO_2 effects (%) on soil NH_4^+ ($n = 44$) and NO_3^- ($n = 30$), and on plant NH_4^+ ($n = 71$) and NO_3^- ($n = 61$) uptake. Error bars, 95% confidence intervals. The elevated CO_2 effect on a response variable was considered significant if the 95% confidence interval did not overlap with 0.

mycorrhizal mediation of decomposition in a N-poor soil, using a model mycorrhizal plant community consisting of AMF growing on roots of *Avena fatua* (*14*) in microcosms (fig. S1). The high levels of CO_2 and N used in our experiment correspond to projected atmospheric CO_2 concentrations and N deposition rates in North America during the 21st century (*17*). We chose *A. fatua*, an annual C_3 grass native to Eurasia, because it has invaded many temperate grasslands and is considered one of the worst weeds in agricultural fields in North America.

After incubation for 10 weeks, AMF enhanced decomposition within hyphae-ingrowth bags ($P < 0.001$, Fig. 1A; also see ^{13}C in fig. S2A). eCO_2 had no impact on total soil C in the absence of AMF (NAMF) ($P > 0.1$, Fig. 1A), but significantly reduced it by 9% in the presence of AMF ($P < 0.01$, Fig. 1A; see ^{13}C in fig. S2A), consistent with the CO_2 stimulation of AMF infection of plant roots ($P < 0.05$, fig. S3A). Notably, the CO_2 effect on AMF-mediated decomposition mainly occurred under the N amendment, with a reduction in total C in hyphae-ingrowth bags of 19% in soil (AMF+S) and 10% in quartz sand (AMF+Q) (Fig. 1A; see ^{13}C in fig. S2A).

Emerging evidence shows that AMF species may differ in their capabilities in acquiring N from decomposing residues (*13*). However, it is unknown whether the nature of AMF species or communities influences the CO_2 effect on residue decomposition. We investigated the effect of three individual AMF species and two AMF assemblages (subplot) on residue decomposition with their host plant *A. fatua* exposed to two atmospheric CO_2 levels (main plot, $n = 4$; 380 versus 580 ppmv) (*16*). One AMF assemblage consisted of three species and the other a total of eight species (table S1).

AMF enhanced decomposition in hyphae-ingrowth cores in comparison with the NAMF ($P < 0.001$, Fig. 1B; see ^{13}C in fig. S2B), particularly under eCO_2. Across five AMF treatments, eCO_2 on average increased AMF infection of plant roots by 28% ($P < 0.05$, fig. S3B) and reduced total C by 15% within hyphae-ingrowth cores ($P < 0.05$, Fig. 1B; see ^{13}C in fig. S2B). The magnitude of the CO_2 effect on decomposition differed among the three individual AMF species ($P < 0.05$), with the high effect found for both *Gigaspora margarita* and *Glomus clarum* and the low for *Acaulospora morrowiae*, but was comparable between the two AMF assemblages ($P > 0.1$). Taken together, these microcosm experiments indicate that CO_2 stimulation of AMF in general enhances organic C decomposition in soils with low N availability.

We also conducted a field study to examine the AMF effect on decomposition in a long-term CO_2 (380 versus 560 ppmv) and O_3 [20 versus 60 parts per billion by volume (ppbv)] experiment (2 × 2 factorial, $n = 4$) in a no-till wheat-soybean system (*16*, *18*). We initiated the long-term experiment in May 2005 and carried out the decomposition study in the wheat season of 2008.

There were no significant O_3 or $CO_2 \times O_3$ effects on any soil microbial parameter (e.g., biomass C and N, fungi/bacteria ratio, and heterotrophic respiration) (*18*), AMF biomass and infection of roots, or organic C decomposition within hyphae- and root-ingrowth bags ($P > 0.05$). However, eCO_2 significantly increased both AMF colonization of fine roots collected from root-ingrowth bags ($P < 0.001$, fig. S3C) and the external AMF biomass as indexed by the biomarker fatty acid 16:1ω5c in the bulk soil ($P < 0.05$, fig. S3D). Concurrently, eCO_2 significantly increased total C losses within hyphae-ingrowth bags across the three sampling points ($P < 0.01$, Fig. 1C; see ^{13}C in fig. S2C). The instantaneous fractional loss rates for C ($k = 1 - X_t/X_0$, where X_t and X_0 are the organic C content at time t and time 0, respectively) induced by the hyphae-ingrowth effect under eCO_2 were 29, 41, and 80% higher than those under aCO_2, respectively, at weeks 5, 10, and 15 (Fig. 1C), indicating that the CO_2 effect on AMF-mediated decomposition did not diminish over time.

To examine whether CO_2 enhancement of AMF-mediated decomposition was accompanied with increased plant uptake of N released from decomposing residues, we determined ^{15}N both in plants and hyphae-ingrowth bags and cores. eCO_2 substantially reduced the total ^{15}N within hyphae-ingrowth bags and cores in the presence of AMF in all three experiments (fig. S4) and increased AMF-mediated plant ^{15}N uptake in the microcosms (fig. S5). These results provide direct evidence of CO_2 enhancement of mycorrhizal N transfer from decomposing organic material to host plants.

We also examined the effect of eCO_2 on soil available N pools [ammonium (NH_4^+) and nitrate (NO_3^-)]. In microcosms where N was limiting and AMF were present, eCO_2 reduced soil NH_4^+ in both experiments ($P < 0.01$, Fig. 2A; $P < 0.05$, Fig. 2B), but did not affect levels of soil NO_3^- ($P > 0.1$, fig. S6D; $P > 0.1$, fig. S6E). In the field where soil N was ample (mainly NO_3^-, fig. S6F), eCO_2 did not affect soil NH_4^+ ($P > 0.1$ for each of three soil layers, fig. S6C) but significantly increased both potential N mineralization (*18*) and soil NO_3^- ($P < 0.05$ for each of three soil layers, Fig. 2C). These results suggest that eCO_2 may differentially affect plant acquisition of soil NH_4^+ and NO_3^-.

We subsequently conducted a meta-analysis (*16*) of 38 studies that quantified the concentrations of soil NH_4^+ and NO_3^- and/or the capacity of plant use of NH_4^+ and NO_3^- under eCO_2 (table S2). These studies encompassed more than 58 species of crop, grass, and tree species (*16*). eCO_2 reduced the capacity of plant NO_3^- use by 16.2% and increased soil NO_3^- by 26.7% (Fig. 2D). By contrast, it had no impact on the capacity of plants to use NH_4^+ but decreased soil NH_4^+ by 7.9% (Fig. 2D). These differential CO_2 effects on soil NH_4^+ and NO_3^- agreed with our results and were consistent qualitatively with recent discoveries of eCO_2 effects on plant N utilization (*19*, *20*). Together, these results suggest that plants under eCO_2 may have to rely more on soil NH_4^+ for N nutrition, and a high demand for NH_4^+ may play a major role in mediating the AMF effect on organic C decomposition.

If CO_2-induced high-plant demand for NH_4^+ is a primary driver in mycorrhizally mediated decomposition, high soil NH_4^+ may partially offset this effect. To test this possibility, we assessed the effect of AMF on decomposition by manipulating soil N transformations with a nitrification inhibitor (dicyandiamide) (*21*) in our long-term field CO_2 and O_3 study in the wheat season of 2011 (*16*). Dicyandiamide had no effect on plant growth and AMF infection of roots ($P > 0.1$). In the no-dicyandiamide control, eCO_2 significantly increased AMF-mediated decomposition ($P < 0.05$, Fig. 3), consistent with the previous field experiment (Fig. 1C). In the dicyandiamide treatment, however, eCO_2 did not affect organic C decomposition in the hyphae-ingrowth bag ($P > 0.1$, Fig. 3), indicating that the nitrification inhibitor largely offset the impact of eCO_2 on AMF-mediated organic C decomposition. These results provide supporting evidence that enhanced plant demand for soil NH_4^+ may be the primary driver for CO_2 enhancement of AMF-mediated decomposition.

Based on this set of investigations, we therefore propose that eCO_2 enhancement of plant N demand prompts plants to invest more C and energy to structures (mainly roots and their associated mycorrhizae) that best garner NH_4^+ from soil (*22*), while stimulating NH_4^+ release from organic materials and reducing NH_4^+ substrate for nitrification (Fig. 4). Two unique AMF properties enable host plants to compete better against nitrifying microbes for NH_4^+ in the fine, discrete decomposing hotspots: (i) external AMF hyphae are at least two orders of magnitude longer and three orders of magnitude thinner than roots (*1*, *15*) and can exploit a much larger soil volume and finer soil microsites; and (ii) AMF possess a special N transfer pathway (*22*, *23*) that can transport soil N from external to internal hyphae and to their hosts preferentially as NH_4^+ with minimal C loss (*23*). Because AMF generally

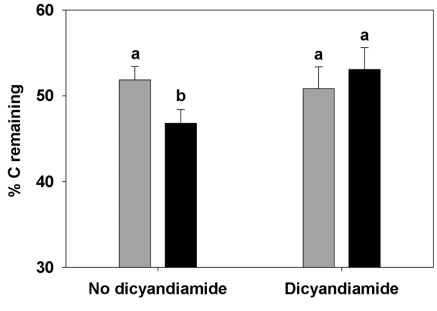

Fig. 3. A nitrification inhibitor (dicyandiamide) offset the CO_2 effect on organic C decomposition within hyphae-ingrowth bags after 10 weeks of incubation in the field. Gray bars, ambient CO_2; black bars, elevated CO_2. Data shown (means ± SEM) are based on the fitted mixed model. The letters a and b represent a significant difference between two CO_2 levels under the no dicyandiamide treatment. The main O_3 effect and the $CO_2 \times O_3$ interaction were not significant in both dicyandiamide and no-dicyandiamide addition treatments ($P > 0.05$).

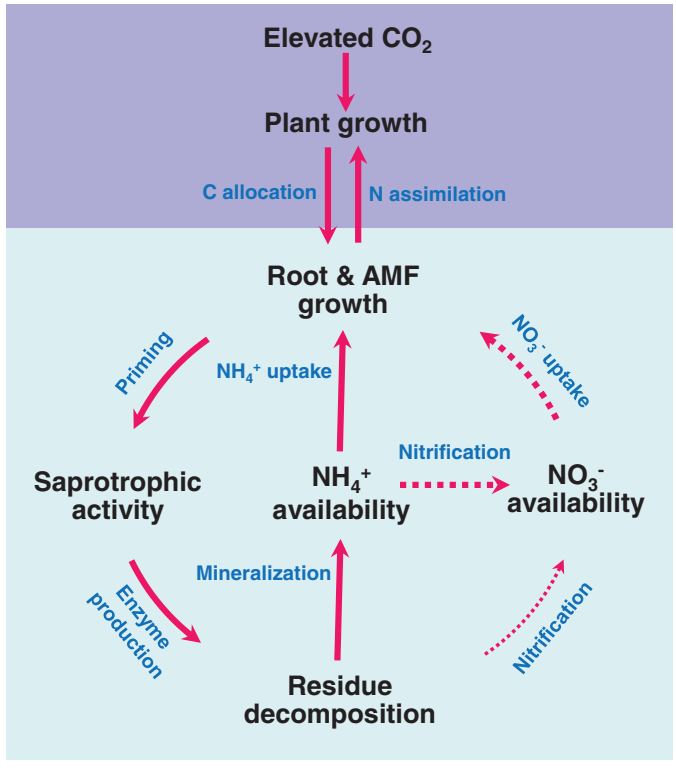

Fig. 4. A conceptual framework of AMF-mediated decomposition driven by CO_2 enhancement of plant N acquisition. CO_2 enhancement of AMF primes residue decomposition and ammonium (NH_4^+) release and optimizes NH_4^+ acquisition while reducing nitrification. CO_2 inhibition of nitrate (NO_3^-) photoassimilation constrains the capacity of plant NO_3^- uptake, prompting plants to rely more on the AMF-mediated pathway of NH_4^+ (and possibly some simple organic N compounds) acquisition. Solid and dashed arrows represent positive and negative CO_2 effects, respectively.

lack saprotrophic capability (*1*), CO_2 enhancement of AMF for N scavenging likely increases decomposition by stimulating (i.e., priming) saprotrophs in soil through three potential mechanisms. First, AMF likely grow preferentially toward (*15*), and thus facilitate saprotrophs' access to, new organic patches (*24*). Second, AMF slowly release labile C for saprotrophs at relatively low concentrations (*3*), likely engendering a larger priming effect on decomposition than roots (fig. S7) (*25*–*27*). And third, rapid removal of newly released NH_4^+ by AMF likely releases saprotrophs from metabolic repression (*28*).

Our findings indicate that CO_2 enhancement of AMF may alter terrestrial ecosystem C dynamics by stimulating decomposition of soil organic C in AMF-active zones. This effect will likely occur in its interplay with other controlling factors such as temperature and plant species composition (*29*). In many agro- or grassland ecosystems where AMF dominate (*1*), but no aboveground C pool with an annual incremental increase exists, CO_2 stimulation of AMF and organic C decomposition will mainly facilitate C turnover belowground, rather than ecosystem C sequestration (*30*). Even in forests with abundant AMF (e.g., tropical forests) (*1*), eCO_2 stimulation of AMF, although creating a transient C sink in plant biomass by facilitating N transfer from soil to plants and partially alleviating N limitation on plants (*31*), is likely to reduce the largest carbon stocks (soil C) in the system. Also, our results suggest that the form, rather than just the total amount, of soil N might play a major role in mediating belowground C turnover and plant N acquisition under eCO_2, thus offering a theoretical foundation for management of microbial N transformations in soil and plant N utilization to facilitate ecosystem C sequestration under future CO_2 scenarios.

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Acknowledgments: We thank F. Chapin III, D. Coleman, Y. Luo, R. Miller, and M. Rillig for valuable comments; M. Gumpertz for advice on statistical analyses; J. Barton, W. Pursley, and E. Silva for technical assistance; and D. Watson and J. Morton for providing mycorrhizal inoculum. L.C. was primarily supported by a fellowship from U.S. Department of Agriculture (USDA)–Agricultural Research Service Plant Science Research Unit (Raleigh, NC) and in part by a USDA grant to S.H. (2009-35101-05351). S.H., L.C. and C.T. conceived experiments 1 to 4. K.O.B. and F.L.B. designed and maintained the long-term CO_2 and O_3 study. H.D.S and T.W.R. contributed to design of experiments 1 and 2. L.C. performed experiments 1 to 3 and the meta-analysis study; and C.T., F.L.B., and L.Z. performed experiment 4. L.C. and S.H. analyzed the data and mainly wrote the manuscript with inputs from all coauthors. The data reported in this paper are deposited in the Dryad Repository (http://dx.doi.org/10.5061/dryad.b7f53).

Supplementary Materials

www.sciencemag.org/cgi/content/full/337/6098/1084/DC1
Materials and Methods
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4 May 2012; accepted 10 July 2012
10.1126/science.1224304

How the Cucumber Tendril Coils and Overwinds

Sharon J. Gerbode,[1,2,3]* Joshua R. Puzey,[4]* Andrew G. McCormick,[5] L. Mahadevan[1,2,4,5]†

The helical coiling of plant tendrils has fascinated scientists for centuries, yet the underlying mechanism remains elusive. Moreover, despite Darwin's widely accepted interpretation of coiled tendrils as soft springs, their mechanical behavior remains unknown. Our experiments on cucumber tendrils demonstrate that tendril coiling occurs via asymmetric contraction of an internal fiber ribbon of specialized cells. Under tension, both extracted fiber ribbons and old tendrils exhibit twistless overwinding rather than unwinding, with an initially soft response followed by strong strain-stiffening at large extensions. We explain this behavior using physical models of prestrained rubber strips, geometric arguments, and mathematical models of elastic filaments. Collectively, our study illuminates the origin of tendril coiling, quantifies Darwin's original proposal, and suggests designs for biomimetic twistless springs with tunable mechanical responses.

The transformation of a straight plant tendril into a helically coiled shape has inspired numerous studies since the 1800s (*1*–*8*), both from mechanistic and functional perspectives. Tendrils serve climbing plants by providing a parasitic alternative to building independently stable structural supports, allowing the plant to wend its way to sunlight and numerous ecological niches (*9*). During climbing, an initially straight tendril first finds and attaches to a support (fig. S1 and movie S1). Once tethered, the tendril coils by forming two oppositely handed helices connected by a "perversion" (Fig. 1, A and B), which was recognized by Darwin as a topological necessity given the clamped boundary conditions at each end of the tendril (*3*). This helical coiling axially shortens the tendril, hoisting the plant toward the attachment point (fig. S1 and movie S1).

Despite the long history of studying tendrils, the basic mechanism of tendril coiling has remained elusive. Historically, experimental studies of diverse tissues [reaction wood (*10*), hypocotyls (*11*), twining stems (*12*, *13*), and leaves (*14*)] have addressed aspects of curvature generation, whereas

[1]School of Engineering and Applied Sciences, Harvard University, Cambridge, MA 02138, USA. [2]Wyss Institute for Biologically Inspired Engineering, Harvard University, Cambridge, MA 02138, USA. [3]Department of Physics, Harvey Mudd College, Claremont, CA 91711, USA. [4]Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA 02138, USA. [5]Department of Physics, Harvard University, Cambridge, MA 02138, USA.

*These authors contributed equally to this work.
†To whom correspondence should be addressed. E-mail: lm@seas.harvard.edu.

Arbuscular Mycorrhizal Fungi Increase Organic Carbon Decomposition Under Elevated CO_2

Lei Cheng, Fitzgerald L. Booker, Cong Tu, Kent O. Burkey, Lishi Zhou, H. David Shew, Thomas W. Rufty, and Shuijin Hu

Science, **337** (6098), .
DOI: 10.1126/science.1224304

A Fungal Culprit to Carbon Loss

In some ecosystems, such as in the layer of soil containing plant roots, fungi, and bacteria, increased levels of CO_2 should stimulate more efficient aboveground photosynthesis, which in turn should promote increased sequestration of organic carbon in soil through the protective action of arbuscular mycorrhizal fungi. However, in a series of field and microcosm experiments performed under elevated levels of CO_2 thought to be consistent with future emissions scenarios, Cheng *et al.* (p. 1084; see the Perspective by Kowalchuk) observed that these fungi actually promote degradation of soil organic carbon, releasing more CO_2 in the process.