

Element interactions limit soil carbon storage

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Rising levels of atmospheric CO₂ are thought to increase C sinks in terrestrial ecosystems. The potential of these sinks to mitigate CO₂ emissions, however, may be constrained by nutrients. By using metaanalysis, we found that elevated CO₂ only causes accumulation of soil C when N is added at rates well above typical atmospheric N inputs. Similarly, elevated CO₂ only enhances N₂ fixation, the major natural process providing soil N input, when other nutrients (e.g., phosphorus, molybdenum, and potassium) are added. Hence, soil C sequestration under elevated CO₂ is constrained both directly by N availability and indirectly by nutrients needed to support N₂ fixation.

global climate change | N₂ fixation | soil organic matter

Numerous studies have reported a surge in plant growth after an abrupt rise in atmospheric CO₂ (1, 2). If increased C assimilation by plants is translated into increased soil organic C content, terrestrial ecosystems might help to mitigate rising anthropogenic CO₂ emissions (3). Simulation models project a wide range of responses of soil C sinks to elevated CO₂. Some models suggest that low nutrient availability will preclude soil C storage (4, 5), whereas others maintain that soil C can accumulate even when nutrient supplies are low (6). Nitrogen fixation, the main source of natural N input in terrestrial ecosystems (7), has been invoked as a process that can potentially diminish N limitation in nutrient-poor systems. Elevated CO₂ has been found to increase N₂ fixation (8), which could provide additional N to support C accumulation in soil. However, N₂ fixation by plants can be limited by the availability of other nutrients such as molybdenum, phosphorus, and potassium (9).

Until now, empirical evidence to evaluate the effect of nutrient availability on soil C storage under elevated CO₂ has been lacking. The effects of nutrient availability and elevated CO₂ are difficult to discern in individual experiments because of high spatial variability in soil C and nutrients and the large amount of C in the soil relative to input rates (10, 11). A quantitative integration of results across multiple studies can overcome some of these problems.

In the current study, we summarized the effect of atmospheric CO₂ enrichment on soil C by performing a metaanalysis on 80 observations from 41 published and unpublished studies. We also summarized the effect of elevated CO₂ on standing root biomass for these studies by using corresponding data for 56 observations on soil C. We divided the studies into three categories of N availability based on N fertilization rates: (i) up to 30 kg·ha⁻¹·yr⁻¹ of N, comparable to maximum atmospheric N depositions in the United States and most of the European Union (12), (ii) between 30 and 150 kg·ha⁻¹·yr⁻¹ of N, typical of extensive agriculture in the United States (13), and (iii) >150 kg·ha⁻¹·yr⁻¹ of N, typical for intensive agriculture in the European Union (13). Similarly, we also evaluated the potential of N₂ fixation to supply extra soil N input under increased CO₂ concentrations by using 92 observations in 25 published and unpublished studies. We compared studies that received additional non-N nutrients and studies that did not. The databases for soil C, root biomass, N₂ fixation, and the results of the meta-

analyses can be found in Data Sets 1–7, which are published as supporting information on the PNAS web site.

Results and Discussion

Elevated CO₂ had no effect on soil C in ecosystems receiving up to 30 kg·ha⁻¹·yr⁻¹ of N (Fig. 1*a*). Soil C accumulation became apparent with increasing inputs of N. Elevated CO₂ increased soil C by 2.1% per year at N additions between 30 and 150 kg·ha⁻¹·yr⁻¹ and by 2.9% per year at N additions >150 kg·ha⁻¹·yr⁻¹. Whether under natural or planted vegetation, in intact or disturbed soils, or with woody or herbaceous species, elevated CO₂ only increased soil C at N additions ≥30 kg·ha⁻¹·yr⁻¹ (Data Set 4). These results, spanning an array of experimental conditions and terrestrial ecosystems, provide powerful evidence that additional N is needed if C is to be stored in soil under elevated CO₂.

Why is the soil C response to elevated CO₂ restricted by N availability? Because an increase in plant growth under elevated CO₂ causes N to accumulate in litter and plant biomass, soil N availability is expected to limit plant growth under elevated CO₂ in the long term without the addition of exogenous N (14). By contrast, N fertilization can sustain increases in plant growth and, thus, soil C input under elevated CO₂ (15, 16). Indeed, soil N availability limited plant growth under elevated CO₂ for the studies contributing to our soil C database; the response of root biomass to elevated CO₂ increased with N additions (Fig. 1*b*). Because new C enters mineral soil mainly through the root system, these results suggest that the effect of N availability on soil C responses to elevated CO₂ were caused by differences in soil C input.

When ecosystems under elevated CO₂ are subject to N stress, soil microbes might mobilize N through increased decomposition of native soil organic matter (17–19). This so-called priming effect could explain why even in unfertilized experiments, elevated CO₂ significantly increases plant biomass (Fig. 1*b*). Yet, as priming simultaneously increases soil N availability but reduces the soil C reservoir, its potential to accommodate soil C storage is probably small. In fact, the increase in root biomass under elevated CO₂ did not lead to C storage in receiving <30 kg·ha⁻¹·yr⁻¹ of N studies (Fig. 1*a*). Thus, although N limitation alone does not always preclude positive plant growth responses to elevated CO₂, the interaction between the C and N cycles in terrestrial ecosystems rapidly constrains the CO₂ effect on soil C contents.

Elevated CO₂ also can promote plant N uptake by growing fine roots and mycorrhizae (20–22). However, the potential for such redistributions of N to accommodate C sequestration is small because the resulting rise in plant growth will further increase readily available C and, therefore, microbial N demand (23). Hence, mechanisms that increase plant N uptake without a net ecosystem gain of N are likely to be constrained by

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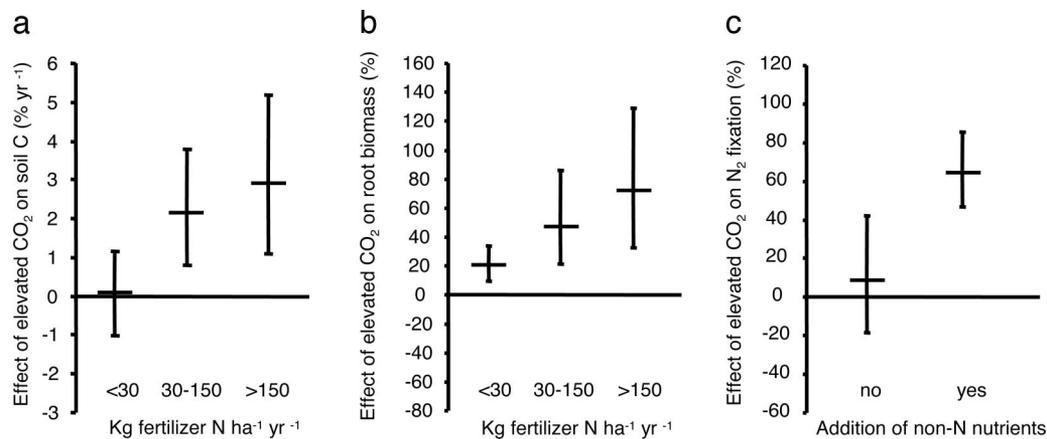


Fig. 1. The effect of elevated CO₂ on soil C contents, root biomass, and N₂ fixation. (a) Change in soil C contents as affected by N fertilization. There is a significant difference between N fertilization classes ($P = 0.02$). The values for <30, 30–150, and >150 kg-ha⁻¹·yr⁻¹ of N are based on 43, 25, and 12 observations, respectively. (b) Change in root biomass as affected by N fertilization. There is a significant difference between N fertilization classes ($P = 0.03$). The values for <30, 30–150, and >150 kg-ha⁻¹·yr⁻¹ of N are based on 29, 17, and 10 observations, respectively. (c) Change in N₂ fixation as affected by nutrient additions. There is a significant difference between studies that received additional non-N nutrients (43 observations) and studies that did not (49 observations) ($P = 0.02$). All observations are weighted by experiment duration and number of replicates. All error bars represent 95% confidence intervals.

ecological stoichiometry (24) and, therefore, self-limiting (25). Thus, the potential for C storage under elevated CO₂ is highest for ecosystems where plant growth is not limited by N availability. In all other cases, other sources of N are needed to support plant growth and sequestration of soil C.

Nitrogen fixation has been suggested to be one of the other N sources (8). However, our metaanalysis shows that elevated CO₂ had no effect on N₂ fixation under conditions representative of most of Earth's terrestrial ecosystems: With no fertilizer additions, with intact soils, and with naturally occurring plant communities, the response of N₂ fixation to elevated CO₂ was indistinguishable from zero (Data Set 6).

Across the entire data set, elevated CO₂ increased N₂ fixation when other nutrients also were added (Fig. 1c). These results suggest that stimulation of N₂ fixation by elevated CO₂ is constrained by the availability of nutrients other than N. In all but one case, non-N nutrient additions included both phosphorus and potassium, which are essential for N₂ fixation (9). Because non-N nutrients were always added in combination (Data Set 3), we cannot test which elements were especially important in releasing N₂ fixation from nutrient limitation. Previous studies have suggested that CO₂ stimulation of N₂ fixation is restricted by the availability of phosphorus (26, 27) and molybdenum (28). Our findings imply that such nutrient constraints are a general feature of N₂ fixation responses to elevated CO₂.

We found no evidence that N fertilization suppressed the response of N₂ fixation to elevated CO₂ (Data Set 6), even though the addition of N fertilizer frequently depresses N₂ fixation (29). Because N addition often occurred in combination with additions of other elements (32 of 45 observations), this result may partly reflect the positive effects of additions of other nutrients overwhelming the negative effects of added N. However, in the subset of observations where we could assess the influence of added N in isolation (in the absence of other nutrient supplements), N fertilization had no effect on the response of N₂ fixation to elevated CO₂ (Data Set 6). Thus, the absence of a response of N₂ fixation to elevated CO₂ without additions of other nutrients was not an artifact caused by N additions.

Elevated CO₂ also increased N₂ fixation in experiments with disturbed soils, an effect comparable in magnitude with that observed by adding non-N nutrients (Data Set 6). Soil disturbance likely operates through its direct effect on nutrient

availability, because it generally decreases soil organic matter contents and liberates nutrients (30). Planted communities showed a significantly stronger CO₂ response for N₂ fixation than natural ecosystems. Yet, all studies on natural communities were performed on intact soils, and none of them received non-N nutrient supplements. The CO₂ response between natural and planted communities did not differ on intact and unfertilized soil (Data Set 6). Thus, planting *per se* did not affect the CO₂ response of N₂ fixation. Together, these results suggest that strong responses of N₂ fixation to elevated CO₂ depend on the availability of non-N nutrients.

The effect of CO₂ on N₂ fixation decreased with experiment duration (Data Set 6; $P = 0.001$). One possible mechanism for this decline is identical to that reducing soil N availability in response to elevated CO₂: After an increase in plant growth, micronutrients required for N₂ fixation accumulate in litter and plant biomass (31). The resulting decrease in non-N-nutrient availability limits the response of N₂ fixation to elevated CO₂ or, in some cases, even turns a stimulation into a suppression (28). In addition, light limitation, often absent in short-term experiments, is likely to become more pronounced over time under elevated CO₂ (32). Together, the dependency of N₂ fixation on supporting nutrients, the lack of a response in natural ecosystems, and the decreasing CO₂ response over time strongly imply that the role of N₂ fixation in providing additional N needed for C storage under elevated CO₂ will be small.

Conclusions

Results presented here show that nutrient limitations of plant growth under elevated CO₂ extend to soil C accumulation and N₂ fixation. Our analysis thus provides empirical corroboration for the largely untested hypothesis that large C accumulations only occur with increased inputs or reduced losses of N (4, 5, 24) and broadens it to non-N nutrients. Together, these conceptual, theoretical, and empirical approaches suggest a limited potential for rapid C storage in the terrestrial biosphere after an increase in atmospheric CO₂.

Methods

Data Collection. We extracted results for soil C contents, root biomass, and N₂ fixation from atmospheric CO₂ enrichment studies conducted in the field, in growth chambers, or in glass houses (Data Sets 1–3). We included observations of the effect

of elevated CO₂ that met several criteria. First, the duration of the experimental CO₂ treatment had to be at least 100 days (the approximate length of a growing season in the temperate zone). Second, means and sample sizes had to be available for ambient CO₂ treatments (between 300–400 ppmV) and elevated CO₂ treatments (450–800 ppmV). Estimates of variance were tabulated when available but were not required for inclusion in the analysis. Third, details of experimental conditions needed to be specified. We only included studies that reported experiment duration, soil sampling depth, plant species, and the type of experimental facility. Studies also needed to indicate N fertilization rates. Most studies applied N additions directly to the soil rather than the canopy. This method of N fertilization is bypassing the possibility of foliar N uptake. Consequently, the lower threshold for N effects on soil C storage and root biomass in our metaanalysis is merely an approximation of atmospheric deposition rates.

Finally, studies needed to indicate whether they involved experiments in pots (i.e., any container with dimensions <1 m) or in ecosystems. We made a distinction between studies on intact and disturbed soils. The latter category included all pot studies, studies on reconstructed soils, and studies that applied tillage during CO₂ enrichment.

Because we examined how effects of elevated CO₂ varied with experimental conditions, we included separate observations of elevated CO₂ effects from a single ecosystem under different experimental treatments (e.g., in multifactorial studies). When studies involved more than one level of CO₂ enrichment, we only included results at the level that is approximately twice the ambient CO₂ concentration.

To increase sensitivity for detecting small effects on soil C, we included only surface soil samples, with a maximum depth of 30 cm. When studies reported soil C contents for multiple depths, we included results that best represented the 0- to 10-cm soil layer. Our analysis focuses on mineral soils; thus, we excluded measurements on forest litter layers, marshes, and bogs. Because soil C accumulates slowly over time and the effects of CO₂ enrichment on soil C are often difficult to discern, we only included measurements after the longest exposure period for each study. Soil C contents were all included in the database as a weight percentage. We converted results reported on an area basis to a weight basis by using soil density data whenever available. When soil density data were not reported, we converted results assuming soil bulk density of 1 g·cm⁻³ in ambient CO₂ and elevated CO₂ treatments. We included root biomass data for the whole sampled soil depth. When root biomass data were reported for multiple years, we included data only from the year closest to the year of the corresponding soil C observation.

All forms of biological N₂ fixation (i.e., free-living and symbiotic bacteria and cyanobacteria) were included. N₂ fixation was determined by acetylene reduction, ¹⁵N dilution, or N contents of plant tissue when atmospheric N₂ was the only available N source. We explicitly examined the temporal dependence of the N₂ fixation response for multiyear studies by using one estimate per treatment combination per year. Although such measurements are not independent, this approach makes it possible to test whether the responses of N₂ fixation change through time. Eliminating nonindependence by restricting the data set to one estimate per ecosystem-treatment combination did not substan-

tially alter the results (Data Set 7). Although this analysis did reveal a stronger response of N₂ fixation in woody compared with herbaceous vegetation, experiments using woody vegetation were dominated by the use of disturbed soils and the addition of nonnitrogenous fertilizers (19 of 24 observations). In the absence of soil disturbance and non-N nutrient supplements, N₂ fixation did not respond to elevated CO₂ in either woody or herbaceous plants (Data Set 7).

Metaanalysis. Data Sets 1–3 were evaluated by using metaanalysis (33). We used the natural log of the response ratio (r = response at elevated CO₂/response at ambient CO₂) as a metric for the response of N₂ fixation and root biomass to elevated CO₂. These results are reported as the percentage change under elevated CO₂ ($(r - 1) \times 100$). The accumulation of soil C in response to an increase in C input follows a logarithmic pattern over time, yet, in the first 5–10 years, accumulation rates will be approximately linear (34). Because the average duration of CO₂ exposure in the metaanalysis was 3.6 years, we assumed linear changes in soil C over time. Accordingly, we used the natural log of the time-adjusted response ratio $r_t = ((r - 1)/y) + 1$ as a response metric, with y as the length of the study in years. We assumed that most of the soil C input occurs during the growing season. Thus, in order to prevent overestimation of annual changes in the soil C pool in short-term studies (<1 year), we used a minimum of $y = 1$. Results are reported as the percentage change per year under elevated CO₂ ($(r_t - 1) \times 100$).

Well replicated and long-term studies provide more reliable estimates of ecosystem CO₂ responses (10). Thus, we weighted the response metrics by replication and experimental duration by using the function $F_c = (n_a \times n_e)/(n_a + n_e) + (y \times y)/(y + y)$, with n_a and n_e as the number of replicates under ambient and elevated CO₂. Using other weighting functions, such as weighting solely by the number of replicates (35) or weighting all studies equally, did not affect the outcome of our analysis (Data Sets 4–7).

The weighting function conventionally used in metaanalyses, i.e., weighting by the inverse of the pooled variance (by using the largest observed variance for missing estimates) yielded similar results. However, this function calculated weights that differed up to three orders of magnitude in size (Data Sets 1–3). By assigning extreme importance to individual observations, average effect sizes were largely determined by a small number of studies. Thus, we favor the alternative weighting functions because they assigned less extreme weights.

We used a mixed model for analysis of all three data sets, based on the assumption that random variation in CO₂ responses occurred between studies (14) and bootstrapping (4,999 iterations) to calculate 95% confidence intervals around mean effect sizes for categories of studies. P values for differences between categories of studies and for correlation with experiment duration were calculated by using resampling techniques incorporated in METAWIN 2.1 (36).

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1. Schimel, D. S. (1995) *Glob. Change Biol.* **1**, 77–91.
2. Mooney, H. A., Drake, B. G., Luxmoore, R. J., Oechel, W. C. & Pitelka, L. F. (1991) *Bioscience* **41**, 96–104.
3. Gifford, R. M. (1994) *Aust. J. Plant Physiol.* **21**, 1–15.
4. Hungate, B. A., Dukes, J. S., Shaw, R., Luo, Y. & Field, C. B. (2003) *Science* **302**, 1512–1513.
5. Rastetter, E. B., Ågren, G. I. & Shaver, G. R. (1997) *Ecol. Appl.* **7**, 444–460.
6. Cannell, M. G. R. & Thornley, J. H. M. (1998) *Glob. Change Biol.* **4**, 431–442.

7. Galloway, J. N., Dentener, F. J., Capone, D. G., Boyer, E. W., Howarth, R. W., Seitzinger, S. P., Asner, G. P., Cleveland, C. C., Green, P. A., Holland, E. A., et al. (2004) *Biogeochemistry* **70**, 153–226.
8. Zanetti, S., Hartwig, U. A., Luscher, A., Hebeisen, T., Frehner, M., Fischer, B. U., Hendrey, G. R., Blum, H. & Nosberger, J. (1996) *Plant Physiol.* **112**, 575–583.
9. Vitousek, P. M., Cassman, K., Cleveland, C., Crews, T., Field, C. B., Grimm, N. B., Howarth, R. W., Marino, R., Martinelli, L., Rastetter, E. B., et al. (2002) *Biogeochemistry* **57**, 1–45.

10. Hungate, B. A., Jackson, R. B., Field, C. B. & Chapin, F. S., III (1996) *Plant Soil* **187**, 135–145.
11. Schlesinger, W. H. & Lichter, J. (2001) *Nature* **411**, 466–469.
12. Holland, E. A., Brasell, B. H., Sulzman, J. & Lamarque, J. F. (2005) *Ecol. Appl.* **15**, 38–57.
13. *FAO Statistical Yearbook 2004* (2004). (Food Agric. Org. U.N., Rome).
14. Finzi, A. C., Delucia, E. H., Hamilton, J. G., Richter, D. D. & Schlesinger, W. H. (2002) *Oecologia* **132**, 567–578.
15. Curtis, P. S. & Wang, X. Z. (1998) *Oecologia* **113**, 299–313.
16. Oren, R., Ellsworth D. S., Johnsen, K. H., Phillips, N., Ewers, B. E., Maier, C., Schafer, K. V. R., McCarthy, H., Hendrey, G., McNulty, S. G., *et al.* (2001) *Nature* **411**, 469–472.
17. Lekkerkerk, L. J.A., Van de Geijn, S. C. & Van Veen, J. A. (1990) in *Soils and the Greenhouse Effect*, ed. Bouwman, A. (Wiley, New York), pp. 423–429.
18. Zak, D. R., Pregitzer, K. S., Curtis, P. S., Teeri, J. A., Fogel, R. & Randlett D. L. (1993) *Plant Soil* **151**, 105–117.
19. Pendall, E., Del Grosso, S., King, J. Y., LeCain, D. R., Milchunas, D. G., Morgan, J. A., Mosier, A. R., Ojima, D. S., Parton, W. A., Tans, P. P. & White, J. W. C. (2003) *Glob. Biogeochem. Cycles* **17**, GB1046.
20. Zak, D. R., Pregitzer, K. S., King, J. S. & Holmes, W. E. (2000) *New Phytol.* **147**, 201–222.
21. Pregitzer, K. S., Zak, D. R., Curtis, P. S., Kubiske, M. E., Teeri, J. A. & Vogel, C. S. *New Phytol.* **129**, 579–585.
22. Klironomos, J. N., Allen, M. F., Rillig, M. C., Piotrowski, J., Makvandi-Nejad, S., Wolfe, B. E. & Powell J. R. (2005) *Nature* **433**, 621–624.
23. Diaz, S., Grime, J. P., Harris, J. & McPherson, E. (1993) *Nature* **363**, 616–617.
24. Sterner, R. W. & Elser, J. J. (2002) *Ecological Stoichiometry* (Princeton Univ. Press, Princeton).
25. Luo, Y., Su, B., Currie, W. S., Dukes, J. S., Finzi, A., Hartwig, U., Hungate, B., McMurtrie, R. E., Oren, R., Parton, W. J., *et al.* (2004) *Bioscience* **54**, 731–739.
26. Niklaus, P. A., Leadly, P. W., Stocklin, J. & Körner, C. (1998) *Oecologia* **116**, 67–75.
27. Edwards, E. J., McCaffrey, S. & Evans, J. R. (2006) *New Phytol.* **169**, 157–167.
28. Hungate, B. A., Stiling, P. D., Dijkstra, P., Johnson, D. W., Ketterer, M. E., Hymus, G. J., Hinkle, C. R. & Drake, B. G. (2004) *Science* **304**, 1291.
29. Woodward, F. I. (1992) *New Phytol.* **122**, 239–251.
30. Haas, H. J., Evans, C. J. & Miles, E. F. (1957) *U.S. Department of Agriculture Technical Bulletin* **1164**, 1–111.
31. Johnson, D. W., Hungate, B. A., Dijkstra, P., Hymus, G., Hinkle, C. R., Stiling, P. & Drake, B. G. (2003) *Ecol. Appl.* **13**, 1388–1399.
32. Grashoff, C., Dijkstra, P., Nonhebel, S., Schapendonk, A. H. C. M. & van de Geijn, S. C. (1995) *Glob. Change Biol.* **1**, 417–428.
33. Hedges, L. V. & Olkin, I. (1985) *Statistical Methods for Metaanalysis* (Academic, New York).
34. Schlesinger, W. H. (1990) *Nature* **348**, 232–234.
35. Adams, D. C., Gurevitch, J. & Rosenberg, M. S. (1997) *Ecology* **78**, 1277–1283.
36. Rosenberg, M. S., Adams, D. C. & Gurevitch, J. (2000) *METAWIN, Statistical Software for Meta-Analysis* (Sinauer, Sunderland, MA), Version 2.