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## CO<sub>2</sub> Elicits Long-Term Decline in Nitrogen Fixation

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Rising atmospheric carbon dioxide  $(C_{a})$ , a product of fossil fuel burning, land-use change, and cement manufacture, is expected to cause a large carbon sink in land ecosystems, partly mitigating human-driven climate change (1). Increasing biological nitrogen fixation with rising  $C_a$  has been invoked as a means to provide the N necessary to support C accumulation (2). As in many short-term experiments (3), we found that  $C_a$  enrichment increased N fixation during the first year of treatment in an oak woodland. However, the effect declined and disappeared by the third year. C<sub>2</sub> enrichment consistently depressed N fixation during the 5th, 6th, and 7th years of treatment. Reduced availability of the micronutrient molybdenum, a key constituent of nitrogenase, best explains this reduction in N fixation. Our results demonstrate how multiple element interactions can influence ecosystem responses to atmospheric change and caution against expecting increased biological N fixation to fuel terrestrial C accumulation.

We investigated the effects of elevated  $C_{a}$ 

ço2

Foliar concentration

(g N m<sup>-2</sup> yr<sup>-1</sup>

N fixation

-40%

60

40

Fig. 1. (A) The relative effect of elevated  $C_a$  on N fixation, calculated as (mean elevated  $C_a$  – mean ambient  $C_a$ )/mean ambient  $C_a \times 100\%$ , for each year of the experiment, showing the consistent decline and reversal of the effect of  $C_a$  on N fixation through time. The decline is best described as relative C<sub>a</sub> effect = [1.514  $\times$   $e^{(-0.658t)}$  – 0.5]  $\times$  100%, where e is the base of the natural logarithm and t is time in years since the beginning of the experiment (in 1996, t = 0). This model provided a substantially better fit ( $r^2 = 0.986$ ) than a linear model ( $r^2 = 0.762$ ). (B) Elevated C<sub>a</sub> reduced the concentration of Fe by 30% and of Mo by 55% in foliage of G. elliottii (Fe, P = 0.043; Mo, P =0.008). Values are means  $\pm$  SEM, n =8 experimental replicates, for the ambient (open) and elevated (solid) C treatments. (C) Correlation between foliar Mo concentration in G. elliottii and N fixation rate for plots exposed to ambient ( $^{\circ}$ ) or elevated ( $^{\bullet}$ )  $C_{a}$ , implicating limitation by Mo as the cause of the suppression of N fixation  $(N \text{ fixation} = 0.25 \ln([Mo]) + 1.49)$  $\dot{r}^2 = 0.586$ ).

on legume N fixation in a stand of scrub-oak vegetation in central coastal Florida, where the leguminous vine *Galactia elliottii* Nutt. occurs naturally (4). During the first year of the experiment, elevated  $C_{\rm a}$  nearly doubled N fixation by *G. elliottii* (Fig. 1A), but this effect disappeared and later reversed ( $C_{\rm a} \times$  time interaction, P = 0.001). Elevated  $C_{\rm a}$  significantly depressed N fixation by *G. elliottii* during the last 3 years of CO<sub>2</sub> exposure (Fig. 1A, repeated measures analysis of variance for 2000 to 2002 only, P = 0.049). During the 7-year period, the relative effect of elevated  $C_{\rm a}$  on N fixation by *G. elliottii* declined exponentially (Fig. 1A).

N-fixing plants are sensitive to light availability (5), so increased shading through greater leaf area could have been responsible for the declining response of *G. elliottii* N fixation to elevated  $C_a$ . However, N-fixing plants also require relatively high concentrations of other nutrients such as phosphorus, iron, and Mo (5). Elevated  $C_a$ increased nutrient accumulation in oak bio-

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mass and in organic forms in the soil (4), potentially reducing their availability to G. elliottii. Elevated  $C_a$  did not alter foliar P in G. elliottii  $(1.19 \pm 0.05 \text{ mg})$  $g^{-1}$  for ambient  $C_a$ versus  $1.08 \pm 0.12^{\circ}$ mg g<sup>-1</sup> for elevated  $C_{\rm a}$ , P = 0.397), but it substantially decreased foliar concentrations of both Mo and Fe (Fig. 1B). This pattern may reflect reduced Mo and Fe concentrations in G. elliottii nodules, where N fixation occurs. Neither leaf area index (r = -0.192, P =0.476) nor foliar Fe (r = 0.024, P =0.843) was significantly correlated with

N fixation by *G. elliottii*, whereas foliar Mo showed a positive, significant relationship (Fig. 1C).

Limitation of N fixation by light and nutrients could both occur, but we submit that the regression analysis provides correlative evidence in favor of the latter and specifically implicates limitation by the availability of Mo. Mo deficiency in Nfixing plants has been documented, particularly in sandy acidic soils (6) similar to the scrub-oak soil studied here (4). By causing soil pH to decline or organic matter to accumulate (both of which increase Mo adsorption to soil particles), elevated  $C_{a}$ could reduce the availability of Mo, causing a systematic decline in N fixation (Fig. 1A). We found that experimental  $C_a$  exposure initially increased but later suppressed N fixation, contrary to the expected response (2, 3). Biogeochemical feedbacks can substantially modify ecosystem responses to global change, and our results underscore the need to broaden the suite of elements typically considered when examining these feedbacks.

## **References and Notes**

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Supporting Online Material

www.sciencemag.org/cgi/content/full/304/5675/1291/ DC1 Materials and Methods

References and Notes

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1996 1997 1998 1999 2000 2001 2002