Unexpected reversal of C₃ versus C₄ grass response to elevated CO₂ during a 20-year field experiment

Peter B. Reich,1,2* Sarah E. Hobbie,3 Tall D. Lee,4 Melissa A. Pastore3

Theory predicts and evidence shows that plant species that use the C₄ photosynthetic pathway (C₄ species) are less responsive to elevated carbon dioxide (eCO₂) than species that use only the C₃ pathway (C₃ species). We document a reversal from this expected C₃-C₄ contrast. Over the first 12 years of a 20-year free-air CO₂ enrichment experiment with 88 C₃ or C₄ grassland plots, we found that biomass was markedly enhanced at eCO₂ relative to ambient CO₂ in C₃ but not C₄ plots, as expected. During the subsequent 8 years, the pattern reversed: Biomass was markedly enhanced at eCO₂ relative to ambient CO₂ in C₄ but not C₃ plots. Soil net nitrogen mineralization rates, an index of soil nitrogen supply, exhibited a similar shift: eCO₂ first enhanced but later depressed rates in C₃ plots, with the opposite true in C₄ plots, partially explaining the reversal of the eCO₂ biomass response. These findings challenge the current C₃-C₄ eCO₂ paradigm and show that even the best-supported short-term drivers of plant response to global climate change might not predict long-term results.

*Corresponding author. Email: preich@umn.edu

The idea that C₃ plants are less limited by ambient atmospheric CO₂ concentrations than C₄ plants, and will thus respond less to increasing CO₂ concentrations, has a long history (1–3) and is deeply embedded in models of past, present, and future vegetation-climate interactions (2–7). The hypothesis has proven useful, if not always entirely predictive, in describing C₃ and C₄ plant distributions (2–4, 8–11) and biomass responses to environmental variation (12–15).

There is strong logic for this hypothesis. C₃ plants, which use the carboxylase enzyme RuBisCO (ribulose-1,5-bisphosphate carboxylase-oxigenase) to fix CO₂ from the air and obtain 3-carbon intermediate, which is then shuttled to the 4-carbon intermediate, which is then used to fix CO₂ until a 3-carbon intermediate is obtained. As a consequence, CO₂ can be fixed. This photosynthetic pathway is often referred to as C₃ photosynthesis.

C₄ plants, on the other hand, use the C₄ photosynthetic pathway. This pathway involves the formation of a 4-carbon intermediate, which is then used to fix CO₂ until a 3-carbon intermediate is obtained. As a consequence, CO₂ can be fixed. This photosynthetic pathway is often referred to as C₄ photosynthesis.

Globally, most plants are C₃; graminoids are the only major functional group (higher biomass on average in plots with one species), and the four-species plots contain all species within each functional group. Annually over 20 years, we sampled both above-ground and below-ground (0 to 20 cm) biomass in each growing season in every plot, and also made an independent measure of fine root production (0 to 20 cm). We also measured in situ soil net N mineralization in every plot for a 1-month period each year just prior to biomass sampling (26, 27). Leaf-level net photosynthetic rates were measured midseason (28) for a subset of these eight grass species in 16 of the 20 years.

Over the 20-year experimental period, total biomass of C₃ grasses became increasingly enhanced by CO₂ exposure, with the reverse true for C₄ grasses (Figs. 1 and 2 and figs. S1 and S2). During approximately the first 12 years (1998–2009), results were as expected (Fig. 1): C₃ plots averaged a 20% increase in total biomass (+136 g/m²) at eCO₂ relative to ambient CO₂, in contrast to C₄ plots that averaged a 1% increase (+12 g/m²). During the subsequent 8 years (2010–2017), the pattern reversed: C₃ plots averaged 2% less (−12 g/m²) and C₄ plots 24% more (+233 g/m²) biomass in eCO₂ than in ambient CO₂ (Fig. 1).

Repeated-measures analyses of variance (Table 1) support these conclusions, which are illustrated for successive 5-year periods in Fig. 2. Significant main effects on total biomass were found for N addition (higher biomass than at ambient N), species richness (higher biomass in plots with four species than in plots with one species), and functional group (higher biomass on average in...
C₄ plots than C₃ plots) (Table 1). Additionally, on average across treatments, biomass of C₃ plots was originally greater than that of C₄ plots, but over time this ranking reversed (interaction of functional group × year, P < 0.0001; Table 1 and Fig. 1). Most germane was the significant functional group × year × CO₂ interaction (P = 0.007; Table 1), showing that C₃ and C₄ functional groups responded differently to eCO₂ over time (Fig. 2). For example, in each of the first two 5-year periods, C₃ grasses increased biomass under eCO₂ by ~20% (+140 g/m²); this declined to a 10% enhancement (+40 g/m²) in years 11 to 15 and a 2% decline in years 16 to 20 (~15 g/m²). In contrast, under eCO₂, biomass of C₄ grasses was reduced by 2% (~23 g/m²) in years 1 to 5, enhanced by ~7% (+60 g/m²) in years 5 to 10 and 11 to 15, and enhanced by 31% (+298 g/m²) in years 16 to 20. These different responses of functional groups to CO₂ and time were unaffected by N treatment (P = 0.76 for functional group × year × CO₂ × N interaction) and were slightly more pronounced in four-species plots than in one-species plots (P = 0.048 for functional group × year × CO₂ × species richness interaction) (Table 1 and fig. S3). Results were generally similar for aboveground and belowground biomass viewed separately, as well as for annual net primary production (estimated as the sum of annual aboveground biomass production and fine root production).

We explored several potential mechanisms for this long-term reversal of C₃ versus C₄ responsiveness to eCO₂, including a temporal switch in leaf-level photosynthetic response, differential CO₂ sensitivity associated with potential climate variation over the 20 years, and potential feedbacks from changing N cycle responses to eCO₂ over time. Measurements of light-saturated net photosynthesis were made for one to three C₃ and one to three C₄ grass species (mean of 2.2) in monocultures in 16 of the 20 years of the study, at all combinations of CO₂ and N treatment. There was no evidence of a shift over time in the enhancement of net photosynthesis as observed for biomass (no interactions of functional group × CO₂ × year; fig. S4). Moreover, there was no correspondence between years when eCO₂ enhancement of net photosynthesis was high and years when eCO₂ enhancement of biomass was high, in either functional group (compare Fig. 1 and fig. S4). Although we lack data for all species in all treatments in all years, the available data provide no evidence to suggest that the rank reversals of biomass responses to eCO₂ were driven by parallel rank reversals in leaf-level photosynthetic responses.

We then asked whether the shifting responsiveness of C₃ versus C₄ grass plots could be related to interannual variation in temperature or rainfall (2–5, 15, 19, 21). Responses of C₃ and C₄ grasses did not depend on year-to-year variations in mean or lagged spring, summer, or growing-season daily average temperature. The only significant effect involved summer rainfall [May to July (MJJ)]; there was a significant (P = 0.0264) interaction of CO₂ × functional group × MJJ rain-
Models that simulate future carbon cycling responses at ecosystem, regional, and global scales assume differing sensitivities of C₃ versus C₄ species to CO₂ based on differences in their photosynthetic physiology (5, 6, 8–11, 17). Although those assumptions have major impacts on vegetation dynamics under varying climate and CO₂ scenarios (8–11, 29, 30), they do not match up well with the dynamic results of this long-term study. Our results thus serve as a reminder that even the best-predicted short-term ecosystem responses to global change can yield mid-term (decades) to long-term (centuries) surprises, as complex responses and interactions may occur over time. Determining whether the mid- to long-term responses demonstrated here are themselves broadly predictable represents a major unmet challenge for experimental and observational studies.

REFERENCES AND NOTES
5. A. B. Harper et al., Geosci. Model Dev. 9, 2415–2440 (2016).
26. See supplementary materials.
P.B.R. wrote the first draft; and all authors jointly revised the manuscript.

**Competing interests:** None.

**Data and materials availability:** The data reported in this paper are available at the Environmental Data Initiative (EDI) (net nitrogen mineralization, DOI 10.6073/pasta/2ac4677a3202904628771d0dd3f75f4a; net nitrogen mineralization, DOI 10.6073/pasta/2ac4677a3202904628771d0dd3f75f4a; aboveground biomass, DOI 10.6073/pasta/8524be900b40a0e7b73458a219d5ed0; belowground biomass, DOI 10.6073/pasta/c00662959002e588597bd7e07dbd16). All other data needed to evaluate the conclusions in the paper are present in the paper or the supplementary materials.

**SUPPLEMENTARY MATERIALS**

www.sciencemag.org/content/360/6386/317/suppl/DC1

**Materials and Methods**

Figs. S1 to S5

Table S1

References (31, 32)

9 January 2018; accepted 21 March 2018

10.1126/science.aas9313
Unexpected reversal of C₃ versus C₄ grass response to elevated CO₂ during a 20-year field experiment
Peter B. Reich, Sarah E. Hobbie, Tali D. Lee and Melissa A. Pastore

Science 360 (6386), 317-320.
DOI: 10.1126/science.aas9313

A short-term trend reversed

Theory and empirical data both support the paradigm that C₄ plant species (in which the first product of carbon fixation is a four-carbon molecule) benefit less from rising carbon dioxide (CO₂) concentrations than C₃ species (in which the first product is a three-carbon molecule). This is because their different photosynthetic physiologies respond differently to atmospheric CO₂ concentrations. Reich et al. document a reversal of this pattern in a 20-year CO₂ enrichment experiment using grassland plots with each type of plant (see the Perspective by Hovenden and Newton). Over the first 12 years, biomass increased with elevated CO₂ in C₃ plots but not C₄ plots, as expected. But over the next 8 years, the pattern reversed: Biomass increased in C₄ plots but not C₃ plots. Thus, even the best-supported short-term drivers of plant response to global change might not predict long-term results.

Science, this issue p. 317; see also p. 263