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

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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 shifted 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 change might not predict long-term results.

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-oxygenase) to fix CO2 from the air and obtain 3-carbon intermediate molecules as the first step in photosynthesis, lose a portion of their fixed CO2 to oxidative photorespiration under present CO2 concentrations because RuBisCO is also an oxygenase (1–3). Thus, C3 plants should exhibit increased leaf-level net photosynthesis as increasing CO2 levels reduce rates of photorespiration and increase rates of carboxylation (1–3). By contrast, in C4 plants, a different enzyme (phosphoenolpyruvate carboxylase) with a high affinity for CO2 and lacking oxygenase activity first incorporates CO2 into a 4-carbon intermediate, which is then shuttled to specialized bundle sheath cells where CO2 is released, resulting in locally high CO2 concentrations. Here, RuBisCO catalyzes carboxylation, but with low rates of photorespiration because of the high CO2 concentrations in the bundle sheath cells. As a result, eCO2 levels in air have little impact on photosynthetic rates for C4 plants (1–3). Globally, most plants are C3 graminoids are the only major group with substantial abundance of both C3 and C4 species (2, 16). Given that C4 grasslands may constitute one-fifth of global terrestrial net primary productivity (16), a better understanding of C4 performance under rising CO2 vis-à-vis C3 grasses is needed for global ecology and for improved ecosystem and Earth system modeling (5–7, 17). C3 and C4 grasses (and sedges) are also distinguished in terms of ecological success by different affinities for temperature, rainfall, and nutrient supply (as well as CO2); as a consequence, they can co-occur or shift their relative abundances depending on the mix of conditions (8–11, 14, 15, 18–20).

Experimental evidence has strongly supported the theoretical prediction that at current CO2 levels, C3 grasses are more CO2-limited than C4 species and thus will respond more to rising CO2. Two influential meta-analyses (12, 13) reported greater stimulation (by factors of 2 to 4) of aboveground biomass by eCO2 for C3 species than for C4 species. One of those publications focused on published studies of graminoids, where plants were mostly grown in pots for less than a year (12), whereas the other focused on free-air CO2 enrichment (FACE) field studies (mostly 3 years or shorter) with plants grown in the ground (13). Another more recent meta-analysis also showed much greater responses of biomass growth for C4 than for C3 plants (22). One of the few longer-term studies comparing C3 and C4 grasses under eCO2 found that C4 species were, surprisingly, apparent “winners” after 3 years of eCO2 and warming (20). However, by 6 years of treatment, the situation had reversed, with C3 species becoming more positively responsive to the simulated global changes and the dominant plant type, in accordance with expectations (22).

In sum, current physiological theory and short- and medium-term studies support the paradigm that C4 species benefit much less from rising CO2 than C3 species. Given both theoretical and empirical support for the differences in eCO2 response of C3 and C4 species, their differential responsiveness to eCO2 comes as close to “accepted fact” as exists in ecology and, as such, is incorporated into many ecosystem and Earth system models (5–6, 8–10). However, it is not known whether findings from short- and medium-term studies apply over ecologically realistic time frames (>10 years) in field settings where complex feedbacks might influence response to eCO2. Understanding long-term responses of C3 and C4 species is especially germane given that the limited available evidence suggests strong nonlinearity of responses of ecosystems to eCO2 over time (23).

Here, we report results from a long-term (20-year) FACE experiment in Minnesota, USA, that support the long-held paradigm for the early part of the experiment but reveal a gradual reversal to a much more positive response to eCO2 by C4 than by C3 grasses. The study uses 88 plots that are components of several different but overlapping global change experiments within the BioCON project (24, 25) and by themselves constitute a fully factorial 2 × 2 × 2 × 2 experiment of CO2 levels (ambient or +180 parts per million), nitrogen levels (ambient or +1 to 2 × 2 experiment of CO2 levels (ambient or +180 parts per million), nitrogen levels (ambient or +4 g N m–2 year–1), species richness (one or four species), and functional group identities (C3 or C4 grasses) (26). Eight species of temperate perennial grasses (four each of C3 and C4) were used in the study (26) and were equally weighted in the original plantings of those 88 plots; that is, there are equal numbers of replicated monocultures of all 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 late 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 eCO2 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

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C₄ plots than in C₃ plots) (Table 1). Additionally, on average across treatments, biomass of C₄ plots was originally greater than that of C₃ 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 one-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 rainfall on the biomass response (table S1); C₄ grasses were slightly more responsive to eCO₂ when rainfall was higher, whereas C₃ grasses were more responsive in low rainfall. These results are inconsistent with C₃ and C₄ grass responses in many studies (2–5, 15, 19). However, MJJ rainfall was only weakly correlated with year, and the CO₂ × year × functional group interaction was significant in the model (\( P = 0.0347 \)) even after accounting for differential responses to rainfall for the two functional groups.
C3 and C4 assemblages. Soil N supply has shaped time was not explained by interannual variation in responses of biomass for the C3 and C4 groups (16-species mixtures) because, as predicted by multi-


Fig. 2. Elevated CO2 effect on total biomass and soil net N mineralization rates in plots comprising C3 grasses and C4 grasses. Left: Mean annual difference in biomass (mean biomass in eCO2 – mean biomass in ambient CO2) for C3 and C4 plots for four time periods during the study (1998–2002, 2003–2007, 2008–2012, and 2013–2017). Right: Mean difference in mean soil N mineralization rate (CO2 – ambient CO2) for C3 and C4 plots for the same four time periods. Each bar represents data pooled across N treatments, and across monoculture and four-species plots (equally weighted), for each functional group (n = 22 plots for each functional group at each CO2 level). Error bars represent SE among years.

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 dynam-

ics 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.

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**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/2ae4671a29200462877d0ed37595fa4; net nitrogen mineralization, DOI 10.6073/pasta/2ae4671a29200462877d0ed37595fa4; aboveground biomass, DOI 10.6073/pasta/8524ab900b40a07eb7a38ba21c9ed2; belowground biomass, DOI 10.6073/pasta/c00662959002e588597d77e0c7d499b). All other data needed to evaluate the conclusions in the paper are present in the paper or the supplementary materials.

**SUPPLEMENTARY MATERIALS**

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Materials and Methods

Figs. S1 to S5

Table S1

References (31, 32)

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