TECHNICAL COMMENT

PLANT ECOLOGY

Comment on “Unexpected reversal of C_3 versus C_4 grass response to elevated CO_2 during a 20-year field experiment”

Julie Wolf* and Lewis Ziska

Reich *et al*. (Reports, 20 April 2018, p. 317) assert that the responses of C_3 and C_4 grass biomass to elevated CO_2 “challenge the current C_3-C_4 [elevated CO_2] paradigm,” but these responses can be explained by the natural history of the experimental plants and soils without challenging this paradigm.

Reich *et al*. (*1*) explain that positive responses of plant biomass to elevated CO_2 have disappeared in C_3 grasses and appeared in C_4 grasses over the 20 years of the BioCON experiment. They assert, as do the authors of the associated Perspective (*2*), that these results challenge current expectations of C_3 and C_4 plant responses to elevated CO_2. Additional context should be made available to qualify this assertion. The pattern documented by Reich *et al*. can be explained by considering the natural history of the experimental plants and soils, without challenging general expectations of C_3 and C_4 grass responses to elevated CO_2 in the absence of other limitations.

The soil at the BioCON experimental field, which was not described in the paper or its supplement, was an excessively drained outwash sand, originally described as a Typic Udipsamment (*3*). When the experiments at the Cedar Creek Ecosystem Science Reserve (including BioCON) were initiated, topsoil was bulldozed away from the experimental field to remove existing savannah vegetation and seedbank. The field was then fumigated with methyl bromide (*4*). Remaining subsoil would have been composed of >90% sand, with little organic matter aside from coatings on sand mineral surfaces. Therefore, despite its 20-year duration, the BioCON experiment documents responses in a disturbed, developing soil. Although results from this experiment might be relevant to agricultural or urbanized soils (*5*), extrapolating to plant communities in mature, undisturbed soils worldwide is problematic (*6*, *7*).

Several publications from BioCON have demonstrated the importance of plant species identity, species richness, and functional group diversity in moderating responses to CO_2 and N enrichment [e.g., (*8*–*10*)], yet Reich *et al*. have used results from monocultures and four-species assemblages of only C_3 or C_4 grasses to make a broad statement about the general responses of C_3 and C_4 grasses to elevated CO_2. Despite variation among species, the C_4 grasses as a group tend to have higher nitrogen use efficiencies than C_3 grasses, reflecting their relatively smaller investment of N in photosynthetic carboxylation enzymes (*11*). Given the individual characteristics of the eight experimental grass species (Table 1) (*12*) and the initial seeding rate of 12 g seed/m^2 for all

Table 1. Traits of the grass species grown in the BioCON experiment (*12*).

Species	Common name	Active growth period	Life span	Growth form	Growth rate	Minimum root depth (inches)	Height at maturity (feet)	Low-growing grass?	Water usage	Drought tolerance	Fertility requirement
					C_4 species						
Andropogon gerardii	Big bluestem	Summer	Long	Bunch	Moderate	20	6	No	Low	High	Low
Bouteloua gracilis	Blue grama	Summer and fall	Moderate	Bunch	Rapid	16	1	No	Medium	High	Low
Schizachyrium scoparium	Little bluestem	Summer and fall	Long	Bunch	Moderate	14	3	No	Low	High	Low
Sorghastrum nutans	Indiangrass	Summer and fall	Long	Bunch	Moderate	24	6	No	Medium	Medium	Low
					C_3 species						
Elymus (Agropyron) repens	Quackgrass	Spring and summer	Moderate	Rhizomatous	Rapid	14	2.6	Yes	Medium	Low	Medium
Bromus inermis	Smooth brome	Spring, summer, and fall	Long	Rhizomatous	Moderate	12	2.5	Yes	Medium	Medium	High
Koeleria cristata (macrocantha)	Junegrass	Spring and fall	Short	Bunch	Rapid	20	1.5	No	High	High	Medium
Poa pratensis	Kentucky bluegrass	Spring, summer, and fall	Long	Rhizomatous	Moderate	10	1.5	Yes	High	Low	High

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plots (*8*), the C_3 grasses would be expected to fill their plots faster than the C_4 grasses, which they did; the C_3 grasses grew greater overall biomass per plot than the C_4 grasses in the first few years (*1*). Short-lived positive responses of the C_3 plant biomass to elevated CO_2 might also be expected, because their higher overall leaf N contents allow for some dilution of N; increased aboveground biomass with diluted N under elevated CO_2 was indeed observed in early years (*13*).

The low fertility and water-holding capacity of the experimental soils, however, would favor the experimental C_4 grass species over time, because their fertility requirements—and, in some cases, their water requirements—are lower than in the C_3 species grown (Table 1). This advantage would not be obvious in the earlier years of the experiment, because of the slower growth rates and longer lifespans of the C_4 species relative to the C_3 plants grown (Table 1), but C_4 biomass would be expected to increase relative to C_3 plants over time in these conditions, with associated increases in organic matter additions to the soil from roots and litter. Eventually, the C_4 plots would accumulate more organic matter, providing carbon substrate for N-mineralizing microbes, as well as increased soil nutrient and water-holding capacity. These changes would alleviate N and H_2O limitations in the C_4 plots relative to the C_3 plots, leading to further enhancements in annual biomass accumulation and nitrogen mineralization rates. Therefore, the observed shifts in relative response to elevated CO_2 over time relate to the differential nutritional requirements also inherent in C_3 and C_4 photosynthetic metabolism, as well as to experimental conditions. Consequently, the observations do not disagree with general expectations of C_3-C_4 dynamics under elevated CO_2 when no other limitations are present.

In addition to methods used to prepare the site before treatment application, the statistical design of the free-air CO_2 enrichment (FACE) arrangement is also important. The authors state that the 88 one- and four-species, C_3-only and C_4-only plots analyzed constitute a fully factorial experiment. In fact, these plots are a subset of a broader experiment where three ambient and three elevated FACE rings provide blocked CO_2 treatments, and N, functional group, and species richness treatments are applied as fully factorial split-plot treatments within the blocks (*10*). The monoculture and four-species plots analyzed in this paper are unevenly distributed among three ambient and three elevated FACE rings. This unbalanced design usually means that the model sum of squares for overall treatment effects is not equal to the sum of individual treatment sums of squares, which precludes straightforward repeated-measures analysis (*14*). The authors do not describe how their statistical analysis addresses these limitations, nor do they mention any multiple-test correction to the *P* values obtained in this and earlier reports of nonindependent response variables over the years of the experiment.

We recognize that long-term (20-year) experiments such as BioCON are invaluable and provide unique information; however, before extrapolating to a broader, ubiquitous inference, attention should be given to both the statistical details and the broader context of the environmental limitations associated with the location. In low-*n* FACE experiments, as described here, underlying variability in soils, particularly nutrient availability, could have an outsized impact on result interpretation [e.g., (*15*)].

The general theory of C_3-C_4 dynamics under elevated CO_2, and its use in the Earth System Models that encode it, is a fundamental aspect of plant biological responses to rising carbon dioxide. Questioning this aspect should be encouraged. However, we would caution that additional research is necessary before the C_3-C_4 dynamic in response to CO_2 is invalidated.

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