TECHNICAL RESPONSE

PLANT ECOLOGY

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

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Wolf and Ziska suggest that soil and species attributes can explain an unexpected 20-year reversal of C_3-C_4 grass responses to elevated CO_2. This is consistent with our original interpretation; however, we disagree with the assertion that such explanations somehow render our results irrelevant for questioning a long-standing paradigm of plant response to CO_2 based on C_3-C_4 differences in photosynthetic pathway.

In a thoughtful consideration of the mechanisms responsible for the unexpected reversal of C_3 versus C_4 grass community responses to elevated CO_2 observed over a 20-year period (*1*), Wolf and Ziska (*2*) make many excellent points. However, they inaccurately represent the interpretations and conclusions of our paper, include at least one key factual error, and come to several conclusions that we believe the evidence does not support.

The thesis of Wolf and Ziska is that our results (*1*) 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." We agree that consideration of the natural history of these plants and soils can help to illuminate the mechanisms and patterns we observed, but nonetheless our long-term results do challenge predictions one would make purely from photosynthetic pathway implications. Moreover, we find curious the authors' assumption that general expectations of C_3 versus C_4 responsiveness to elevated CO_2 have historically been framed as relevant only when no other limitations are present (which in any case never or almost never occurs); instead, such differences between C_3 and C_4 species are considered to hold generically, across the full spectrum of limitations. Additionally, we dispute that we extrapolated these results to grasslands globally; instead, we stated (*1*) in the penultimate sentence that "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." Our final sentence further stated that "Determining whether the mid- to long-term responses demonstrated here are themselves broadly predictable represents a major unmet challenge for experimental and observational studies." Both statements suggest that extrapolating from short-term physiology to long-term biogeochemistry may be problematic; however, they do not suggest that the specific responses of our experimental communities are repeatable elsewhere.

Wolf and Ziska introduce three topics they think did not receive needed attention in our original paper: (i) the level of soil disturbance during establishment of the experiment, and thus the relevant ecological context for our study; (ii) the nature of the plant species used in the experiment, and what that might mean for interpreting the results; and (iii) whether elements of the statistical design and/or analyses were of concern. We address each in turn.

As Wolf and Ziska note, the soil at the experimental site is sandy, nutrient-poor and very well drained (*3*). It is not true, however, that "topsoil was bulldozed away from the experimental field to remove existing savannah vegetation and seedbank." The experiment was established in an old field and topsoil was not removed; prior to planting, the experimental area was tilled to a depth of 25 cm, fumigated with methyl bromide to eliminate seeds of undesired plant species, and the soil reinoculated with unfumigated soil suspended in water (*4*, *5*). It is true that the experiment is situated on disturbed soil (initially to institute agriculture long ago, and 20 years ago to establish the experiment). But much of the world's grasslands are also disturbed, having experienced changes in grazer identity, fire regime, woody species control, cropping, and/or pasture management that also directly or indirectly influence soil properties. More important in our view is asking whether and how CO_2-mediated feedbacks to the nitrogen cycle that occurred at this site might occur elsewhere in poor (or, for that matter, rich) soils. At present, we have little basis for knowing the answer. Thus, although we agree that "extrapolating to plant communities in mature, undisturbed soils worldwide is problematic," we believe that our results are highly relevant for soils with chronic nitrogen limitations, whether disturbed or undisturbed, akin to much of the world's grasslands, and much less relevant to agricultural systems, where nutrient inputs can easily swamp plant-driven feedbacks.

Wolf and Ziska discuss differences in perennial C_3 and C_4 grasses that may well be related to the different temporal patterns shown by the two functional groups (in both CO_2 levels) and suggest that these differences led to the shifting responses to CO_2 over time. We agree that a number of aspects of physiology and life history likely led to these C_3 grasses having high biomass in early years of the experiment, which then declined, and to the C_4 grasses slowly (if erratically) ramping up their biomass over time. What is unclear is whether these successional differences between the C_3 and C_4 plants influenced their responses to CO_2. Indeed, in future work, we plan to test a suite of interrelated hypotheses regarding whether plant-soil feedbacks and differences in resource use efficiency among C_3 and C_4 species contributed to the differing temporal patterns of effects

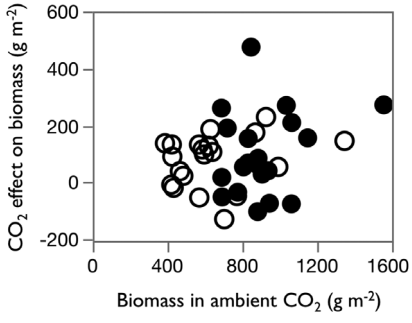

Fig. 1. The CO_2 effect on biomass in plots comprising C_3 grasses and C_4 grasses in relation to total biomass in ambient CO_2. Biomass (aboveground + belowground, 0 to 20 cm) in ambient CO_2 is shown for each year from 1998 to 2017 for plots comprising C_3 grasses (open circles) and C_4 grasses (solid circles); the CO_2 effect on biomass is expressed as the difference in biomass between elevated and ambient CO_2. Each point 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 CO_2 level) in each year. There was no relationship ($P > 0.10$) between the CO_2 effect size and the ambient biomass for all data pooled or for either functional group alone.

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of elevated CO_2. Here, we evaluated one related question that we pursued in the early stages of analyzing the data prior to publication: whether the relative "vigor" (as measured by biomass in ambient treatment) of the C_3 versus C_4 grasses influenced their response to elevated CO_2. One might plausibly hypothesize that when a community is growing most vigorously, it might have the greatest capacity to use extra resources, such as elevated CO_2. The data, though, provide no evidence that in years with high biomass accumulation under ambient CO_2, the biomass response to elevated CO_2 was higher (for either the C_3 or C_4 group, alone or together) (Fig. 1). Nonetheless, we share the belief of Wolf and Ziska that ecosystem-scale changes driven by differences among C_3 and C_4 species could play a role in the observed and future responses to elevated CO_2. It makes no sense, however, for Wolf and Ziska to lodge this as a criticism of our paper, as the mechanisms they propose (involving leaf nitrogen status and feedbacks to soil nitrogen biogeochemistry) are consistent with our observations and interpretations. Thus, if ecosystem-scale temporal changes do play a role, they would likely help to explain and support our unexpected result, rather than suggest that we can continue to rely on the notion that C_4 grassland communities would be generally unresponsive to CO_2 while those dominated by C_3 grasses would generally show biomass enhancement. We hope that by elucidating the underlying mechanisms in the future, we will be in a better position to evaluate the generality of the responses we observed.

Wolf and Ziska also question three aspects of our statistical approach. First, we wish to clarify that despite their implication to the contrary, the 88 plots do in fact constitute a complete factorial of species number, functional group, CO_2 treatment, and N treatment. Second, it is true that plot-level replication is uneven among species richness levels (there are a total of three four-species plots and eight one-species plots per functional group at each unique CO_2 and N level), and Wolf and Ziska expressed concern that the unbalanced design produced sums of squares that are not straightforward to interpret. We did not explain explicitly in the original paper that the analyses we made are insensitive to this. The analyses were made in a statistical program (JMP 13.1) using a mixed model (with both fixed and random effects) that deploys a maximum likelihood approach; it does not calculate sums of squares, but partitions the variance and then uses that to calculate *F* statistics. It is robust to unbalanced sample sizes; hence, this concern is unwarranted. The third statistical concern involved our lack of multiple-test corrections to the *P* values. This concern is not applicable, as we only focused on the main effects and interactions from the analysis shown in table 1 of (*1*) and did not compare specific levels of factors. Regardless, visual examination and further analyses of the data shown in figure S2 of (*1*) support our conclusion. We tested whether the effect size (for both biomass and net N mineralization) was significantly related to year (a continuous variable), functional group, or their interaction. We found no significant main effects of year or functional group, but we did find significant year × functional group interactions ($P < 0.0001$ and $P = 0.0035$ for biomass and net N mineralization, respectively), supporting the interpretation that the response to elevated CO_2 changed over time and did so in opposing fashion for the C_3 and C_4 grass groups.

In summary, the questions raised about statistics (*2*) are not of concern, and we disagree with Wolf and Ziska about the appropriate context within which to view the experiment, but we agree that finding the appropriate context for field experiments is always challenging and should be done carefully. Perhaps most intriguing, Wolf and Ziska highlight important aspects of the way in which these North American grassland ecosystems change over time. Such changes might plausibly contribute to the reversal of responses to elevated CO_2 of these two functional groups over time such as we observed; but if they did, this would be entirely consistent with our interpretation that over time, plant-soil feedbacks can outweigh photosynthetic pathway differences in driving ecosystem responses to CO_2. Ecosystems change over time in complex ways that we are only beginning to understand. Even if we could fully explain why our experimental communities responded as they did over 20 years, which we cannot yet do, it would remain a post hoc explanation of an unpredicted and unexpected result. We hope for more unexpected results among the thousands of observational, experimental, and modeling studies researchers are engaged in globally, as such results often do a better job of illuminating gaps in our thinking and data than results that conform to expected outputs, and which thus may hide errors in our thinking, data, and modeling regarding underlying mechanisms (*6*).

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