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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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Nie and colleagues suggest a key role for interannual climate variation as an explanation for the temporal dynamics of an unexpected 20-year reversal of biomass responses of C_3-C_4 grasses to elevated CO_2. However, we had already identified some climate-dependent differences in C_3 and C_4 responses to eCO_2 and shown that these could not fully explain the temporal dynamics we observed.

Using some new analyses, Nie and colleagues (*1*) suggest that growing season temperature and rainfall can explain much more of the unexpected 20-year reversal of C_3 versus C_4 grass community responses to elevated CO_2 than our analyses and interpretation concluded (*2*). They based their analyses on statistical models using 3-year running averages of the effect of eCO_2 on total biomass (i.e., the average difference between ambient and enriched CO_2 across all C_3 or C_4 plots), which they compared to the 3-year running average of growing season (May–Sept) rainfall and temperature.

We question whether using 3-year running averages [which we included solely for visualization purposes in (*2*)] is the best way to test for interannual climate variation interactions with the elevated CO_2 treatment; we believe that at the very least, using annual data makes more sense for such examinations. Moreover, we believe that using all data (i.e., 88 plots for all 20 years, $n = 1760$) in a mixed model [as in (*2*)] makes the best use of all available information; whereas Nie *et al.* used a data set of $n = 36$, comprising just the 18 values representing the 3-year running averages of effect size for each of the C_3 and C_4 groups. As two-thirds of the data for each 3-year running average of effect size is shared with both the prior and subsequent years, there is considerable lack of independence of such data across years.

Additionally, Nie *et al.* chose to use climate data from the Minneapolis–St. Paul International Airport rather than data available from Cedar Creek. The airport is more than 60 km south of the experimental site, averaged >1.5°C warmer for the growing seasons in question, and most problematic, had only moderate correlations ($R^2 \approx 0.5$) for May–Sept rainfall and temperature with the same metrics at the experimental site, meaning they share only roughly 50% of the same information. Given that Cedar Creek lies outside of the urban heat island while the airport lies within it (*3*), the relatively fine-scale spatial variability in rainfall, and the availability of data on site, use of the alternate data from the southern Twin Cities metropolitan area is not warranted, in our view. Note also that Nie *et al.* use growing season temperature and rainfall defined as May–September values (except in their figure 2 where they used MJJ rainfall and unspecified temperature data), whereas in (*2*) we used summer temperature and rainfall defined as May–July (MJJ); our rationale was that biomass harvests and net nitrogen mineralization assays were completed by very early August each year, such that the three prior months were a reasonable metric for assessing climate sensitivity of CO_2 responses. We also used MMJ rainfall measured at the experimental site, rather than at the Cedar Creek weather station 2 km away, for years when it was available.

Regardless of the appropriateness of the approach used, Nie *et al.* assert that potential collinearity among explanatory variables might have masked the true effects of climate on biomass responses in our analyses and suggest that their analysis with only two independent variables (growing season rainfall and temperature) avoids such potential problems. They found that response of C_4 biomass to eCO_2 was positively correlated with both growing season rainfall and temperature (May–September) and response of C_3 biomass to eCO_2 was negatively correlated with growing season temperature. However, when we ran similar analyses to Nie *et al.* using annual biomass differences (between ambient and elevated CO_2) and rainfall and temperature data from the experimental site, rather than 3-year running averages of biomass from the experiment and 3-year running average climate data from the Minneapolis–St. Paul International Airport, we did not find any significant (P < 0.10) relationship with MJJ or May–September temperature for either functional group (Fig. 1). We also did not find any significant (P < 0.10) relationship with MJJ or May–September rainfall for the C_3 grasses (Fig. 1). We did find a marginally significant positive (P = 0.08) relationship of C_4 biomass response to eCO_2 with May–September rainfall (Fig. 1). However, the simple bivariate fit of C_4 biomass response to eCO_2 versus May–September rainfall using annual data (and local climate data) was weaker (R^2 = 0.16, R^2 adjusted = 0.12) than when using the 3-year running average data used by Nie *et al.* (R^2 = 0.32). Thus, annual data do also suggest some degree of dependency of C_4 biomass response to eCO_2 to growing season rainfall (May–Sept), similar to that previously reported in relation to MJJ rainfall in (*2*), albeit not as strongly as suggested by the analyses of Nie *et al.* However, annual data provide no support for any such dependency for either functional group on summer or growing season temperature.

Moreover, we had tested for collinearity among explanatory variables in our original analyses and found it to be extremely modest; although we did not report these results in (*2*), we did point out that MJJ rainfall was only weakly correlated with year as a continuous variable (*2*). Thus, we were able to independently assess the effects of MJJ rainfall and year on the effects of CO_2 on C_4 versus C_3 biomass [table S1 of (*2*)]. As reported in (*2*), we noted a significant (P = 0.0243) interaction of CO_2 × functional group × MJJ rainfall on the biomass response [table S1 of (*2*)]; C_4 grasses were more responsive to eCO_2 when rainfall was higher, whereas C_3 grasses were more responsive in low rainfall (the same conclusion Nie *et al.* draw from their analysis). However, we also found that the CO_2 × year × functional group interaction was significant (P = 0.0347) even after accounting for differential responses to rainfall for the two functional groups by including rainfall and rainfall interactions in the model [table S1 of (*2*)]. Thus, despite differential sensitivity to eCO_2 as a function of summer rainfall, the reversal of responsiveness of C_3 and C_4 plots to eCO_2 over time was not explained by interannual variation in precipitation. Including temperature in the above model [which we had tested for but did not report in (*2*)] did not alter the results and there were no interactions involving temperature and eCO_2 response for either functional group. Thus, the reversal of responsiveness of C_3 and C_4 plots to eCO_2 over time was not explained by interannual variation in temperature.

In summary, we do not believe that the approach taken by Nie and colleagues is sufficiently robust to overturn our conclusions that C_3 and C_4 group responses to CO_2 were differentially sensitive to summer rainfall, but that those differences did not cause the longitudinal shift over time in responses of the two groups. Figure 2 of Nie *et al.* is consistent with our interpretation in (*2*); C_4 grasses responded more positively to eCO_2 in moist than dry years, and late in the experiment than early in the experiment. Nie *et al.* suggest visually that the average of 1°C warmer summers after 2010 made the C_4 grass response to eCO_2 during those moist 8 years stronger than during the moist 7 years from 1999–2005. In a full model that includes year, local summer temperature, and local summer rainfall, we find no evidence that responses to eCO_2 were greater in C_4 grasses in warmer summers ignoring summer rainfall (i.e., there was no CO_2 × functional group × MJJ temperature interaction) or in warmer summers that also had higher summer rainfall (i.e., there was no CO_2 × functional group × MJJ temperature × MJJ rainfall interaction). Thus, although their ideas are intriguing, our analyses do not provide evidence to support them.

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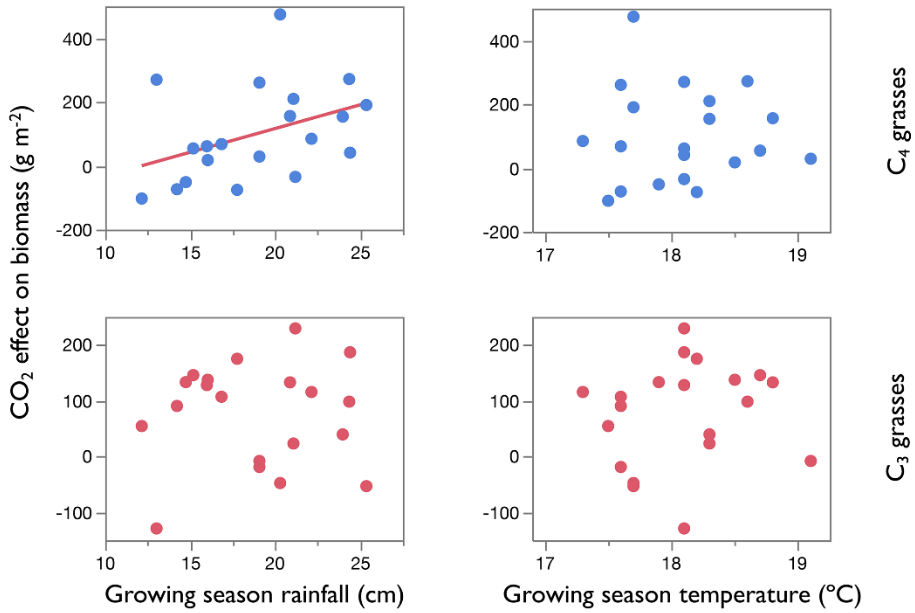

Fig. 1. Bivariate relationships between the CO_2 effect on total C_3 and C_4 biomass and growing season climate using annual biomass and climate data. CO_2 effect size = biomass under eCO_2 – biomass under ambient CO_2. Growing season is defined as May–September. Biomass data match those in figure S1 of (*2*). Note, unlike in (*1*), climate data are from Cedar Creek, not the Minneapolis–St. Paul International airport. The relationship between the CO_2 effect size and temperature was not significant for either the C_3 group ($P = 0.55$, $R^2 = 0.02$) or the C_4 group ($P = 0.98$, $R^2 = 0.00$). The relationship between the CO_2 effect size and rainfall was not significant for the C_3 group ($P = 0.88$, $R^2 = 0.00$) and was marginally significant for the C_4 group ($P = 0.076$, $R^2 = 0.16$).

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