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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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Reich *et al.* (Reports, 20 April 2018, p. 317) reported that elevated carbon dioxide (eCO_2) switched its effect from promoting C_3 grasses to favoring C_4 grasses in a long-term experiment. We argue that the authors did not appropriately elucidate the interannual climate variation as a potential mechanism for the reversal of C_4-C_3 biomass in response to eCO_2.

Reich *et al.* (*1*) presented results of a long-term free-air CO_2 enrichment experiment. The results showed that elevated CO_2 (eCO_2) favored C_3 grasses rather than C_4 grasses during the first 12 years; however, the pattern reversed during the subsequent eight years. It appears that their observations regarding the changes in C_4-C_3 grasses under eCO_2 condition did not reflect the effects of inter-annual variations in the ambient rainfall and temperature during the 20-yr experimental period, leading to uncertainties in their results.

The effect of eCO_2 on plant biomass largely depends on the ambient rainfall and temperature (*2*, *3*). However, Reich *et al.* (*1*) found that the responses of C_4 and C_3 grasses to eCO_2 had negligible dependence on these important climatic factors, determined by estimating the effects of multiple variables on C_4-C_3 biomass with repeated-measures analysis. According to the Cedar Creek weather data from Fort Snelling near the Saint Paul International Airport, the ambient total rainfall (316–722 mm) and average temperature (18.6–21.4°C) during the growing season had considerable inter-annual variations during the 20-yr experimental period. Using generalized linear models, we found that both the growing-season rainfall and average temperature positively correlated with the effect of CO_2 on C_4 biomass and the growing-season average temperature negatively correlated with the effect of CO_2 on C_3 biomass (Fig. 1). Without potential collinearity among the explanatory variables and order effects of repeated-measures analysis, our analysis is more appropriate to estimate the effect of individual variable on the response of C_4 or C_3 biomass to eCO_2 with an accurate and interpretable predictor.

The change in C_4 biomass showed a sharp decrease from 2005 to 2008 (Fig. 2), and the C_3 biomass also reached the lowest level during this period (*1*). Water stress during summer might have led to the decrease in biomass because summer rainfall during these dry years was about 53% less than the average of other years (Fig. 2). After these dry years, eCO_2 favored C_4 but not C_3 grasses. Besides the asymmetric changes in net nitrogen mineralization rates between C_4 and C_3 soils as suggested by Reich *et al.* (*1*), we offer two other possible mechanisms for the "winner"—C_4 grasses. First, increased growing-season average temperature might favor C_4 than C_3 grasses under eCO_2 condition. The growing-season average temperature significantly increased by approximately 0.98°C before and after the dry years (Fig. 2; $t = -3.6$; $P < 0.01$). By the two-way ANOVA with CO_2 (ambient CO_2 versus eCO_2) and average growing-season temperature (before versus after the dry years) as fixed factors to determine the effects of eCO_2 and temperature on the 3-yr moving averaged C_4 biomass, we found that increased growing-season temperature might interact with eCO_2 to affect $\log_{10}$-transformed C_4 biomass ($F = 4.4$; $P < 0.05$). As suggested by other studies and as shown in Fig. 1, the warm-season C_4 grasses can grow better than C_3 grasses under higher temperature conditions (*4*), and can enhance their sensitivity to eCO_2 with increasing temperature when soil moisture content is not limited (*4*–*6*). Second, C_3 grasses as cool season species lose their positive responses to eCO_2 with increase in the ambient temperature as shown in Fig. 1.

Understanding the directions and magnitudes of responses of C_4 and C_3 grasses to eCO_2 is crucial in modeling carbon-climate feedbacks. It is difficult to predict the changes in plant biomass dynamics in an intricate ecosystem based only on the photosynthetic pathways. Several studies have shown that the relative effects of eCO_2 on the biomass of C_4 and C_3 grasses are highly influenced by soil water availability and temperature (*2*–*6*). We argue that the interpretation of the biomass data would be more meaningful by appropriately considering the effects of inter-annual variations in the ambient rainfall and temperature. Our analysis and interpretation of the biomass data provides insights different from those of Reich *et al.* (*1*), but we fully support their call for long-term experiments.

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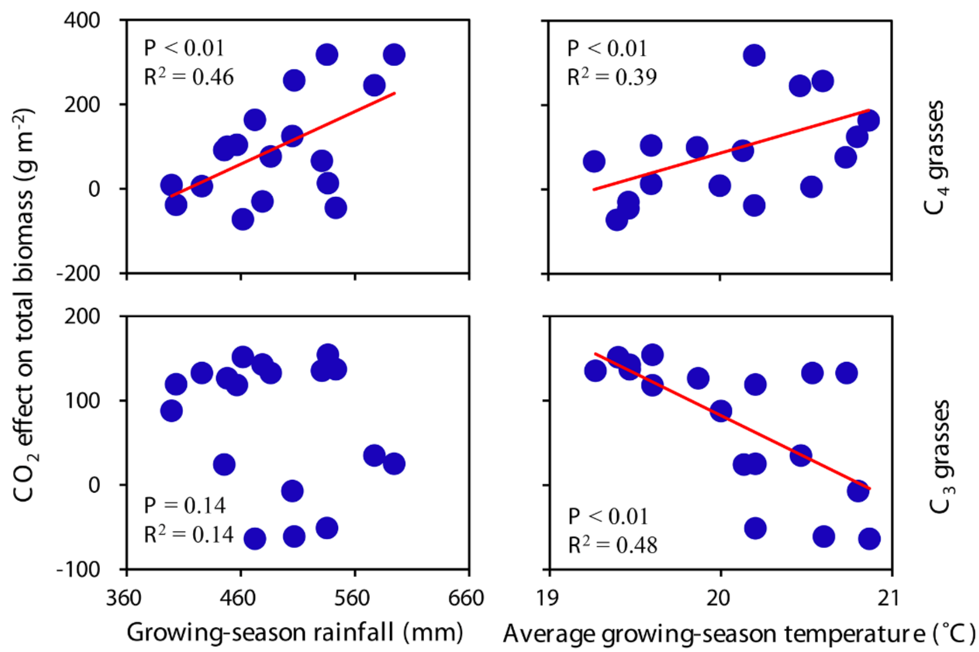

Fig. 1. Relationships between the CO_2 effect on total C_4-C_3 biomass and growing-season climate. The biomass data are from the measurements of Reich *et al*. (*1*). CO_2 effect size = biomass under eCO_2 condition – biomass under ambient CO_2 condition. The Cedar Creek weather data are from Fort Snelling near the Saint Paul International Airport (www.wunderground.com/history/airport/KMSP). The biomass and weather data are shown as 3-yr moving averages centered over the middle of each 3-yr group. The relationships between CO_2 effect size and climatic factors were analyzed using generalized linear models (CO_2 effect size – temperature + rainfall; family = Gaussian; link = identity). The partial R^2 of each climatic factor was obtained using the rsq. partial function with the rsq package in the R version 3.2.2.

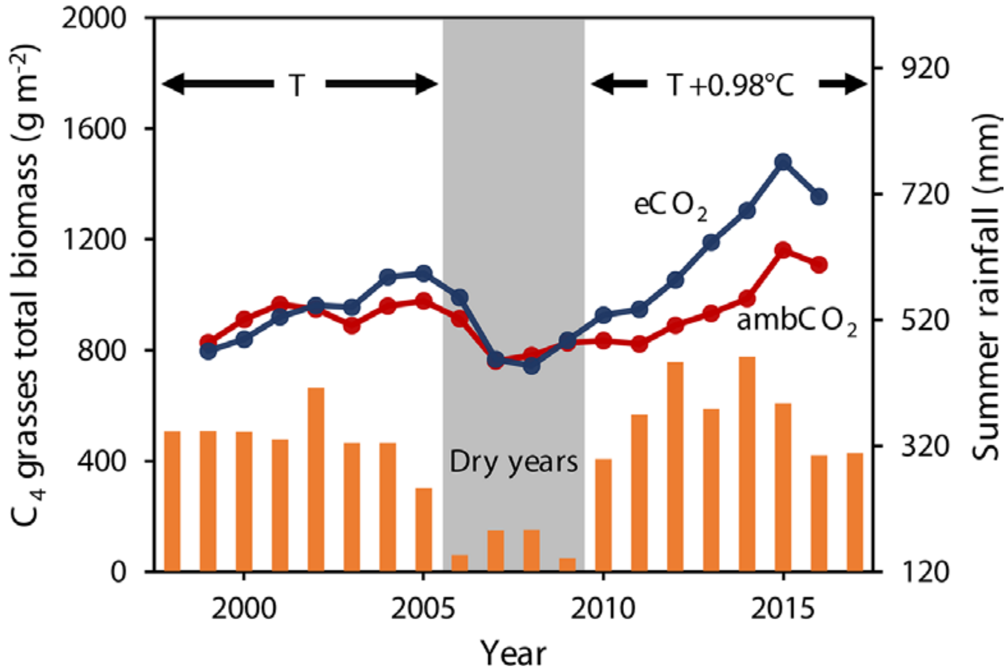

Fig. 2. Inter-annual trajectories of C_4 total biomass at the ambient (red) and elevated CO_2 (blue) levels and summer rainfall (orange). The Biomass data from the measurements of Reich *et al*. (*1*) are shown as 3-yr moving averages centered over the middle of each 3-yr group. The Cedar Creek weather data are from Fort Snelling near the Saint Paul International Airport.

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