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# Comment on “Unexpected reversal of C<sub>3</sub> versus C<sub>4</sub> grass response to elevated CO<sub>2</sub> during a 20-year field experiment”

Ming Nie,<sup>1\*</sup> Junyu Zou,<sup>1†</sup> Xiao Xu,<sup>1†</sup> Chao Liang,<sup>2</sup> Changming Fang,<sup>1</sup> Bo Li<sup>1</sup>

<sup>1</sup>Ministry of Education Key Laboratory for Biodiversity Science and Ecological Engineering, Coastal Ecosystems Research Station of the Yangtze River Estuary, Fudan University, 2005 Songhu Road, Shanghai 200438, China. <sup>2</sup>Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, China.

\*Corresponding author. Email: mnie@fudan.edu.cn

†These authors contributed equally to this work.

Reich *et al.* (Reports, 20 April 2018, p. 317) reported that elevated carbon dioxide (eCO<sub>2</sub>) switched its effect from promoting C<sub>3</sub> grasses to favoring C<sub>4</sub> 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<sub>4</sub>-C<sub>3</sub> biomass in response to eCO<sub>2</sub>.

Reich *et al.* (1) presented results of a long-term free-air CO<sub>2</sub> enrichment experiment. The results showed that elevated CO<sub>2</sub> (eCO<sub>2</sub>) favored C<sub>3</sub> grasses rather than C<sub>4</sub> 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<sub>4</sub>-C<sub>3</sub> grasses under eCO<sub>2</sub> 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<sub>2</sub> on plant biomass largely depends on the ambient rainfall and temperature (2, 3). However, Reich *et al.* (1) found that the responses of C<sub>4</sub> and C<sub>3</sub> grasses to eCO<sub>2</sub> had negligible dependence on these important climatic factors, determined by estimating the effects of multiple variables on C<sub>4</sub>-C<sub>3</sub> 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<sub>2</sub> on C<sub>4</sub> biomass and the growing-season average temperature negatively correlated with the effect of CO<sub>2</sub> on C<sub>3</sub> 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<sub>4</sub> or C<sub>3</sub> biomass to eCO<sub>2</sub> with an accurate and interpretable predictor.

The change in C<sub>4</sub> biomass showed a sharp decrease

from 2005 to 2008 (Fig. 2), and the C<sub>3</sub> 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<sub>2</sub> favored C<sub>4</sub> but not C<sub>3</sub> grasses. Besides the asymmetric changes in net nitrogen mineralization rates between C<sub>4</sub> and C<sub>3</sub> soils as suggested by Reich *et al.* (1), we offer two other possible mechanisms for the “winner”—C<sub>4</sub> grasses. First, increased growing-season average temperature might favor C<sub>4</sub> than C<sub>3</sub> grasses under eCO<sub>2</sub> 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<sub>2</sub> (ambient CO<sub>2</sub> versus eCO<sub>2</sub>) and average growing-season temperature (before versus after the dry years) as fixed factors to determine the effects of eCO<sub>2</sub> and temperature on the 3-yr moving averaged C<sub>4</sub> biomass, we found that increased growing-season temperature might interact with eCO<sub>2</sub> to affect log<sub>10</sub>-transformed C<sub>4</sub> biomass ( $F = 4.4$ ;  $P < 0.05$ ). As suggested by other studies and as shown in Fig. 1, the warm-season C<sub>4</sub> grasses can grow better than C<sub>3</sub> grasses under higher temperature conditions (4), and can enhance their sensitivity to eCO<sub>2</sub> with increasing temperature when soil moisture content is not limited (4–6). Second, C<sub>3</sub> grasses as cool season species lose their positive responses to eCO<sub>2</sub> with increase in the ambient temperature as shown in Fig. 1.

Understanding the directions and magnitudes of responses of C<sub>4</sub> and C<sub>3</sub> grasses to eCO<sub>2</sub> is crucial in modeling carbon-climate feedbacks. It is difficult to predict the changes in plant biomass dynamics in an intricate ecosys-

tem based only on the photosynthetic pathways. Several studies have shown that the relative effects of eCO<sub>2</sub> on the biomass of C<sub>4</sub> and C<sub>3</sub> 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.

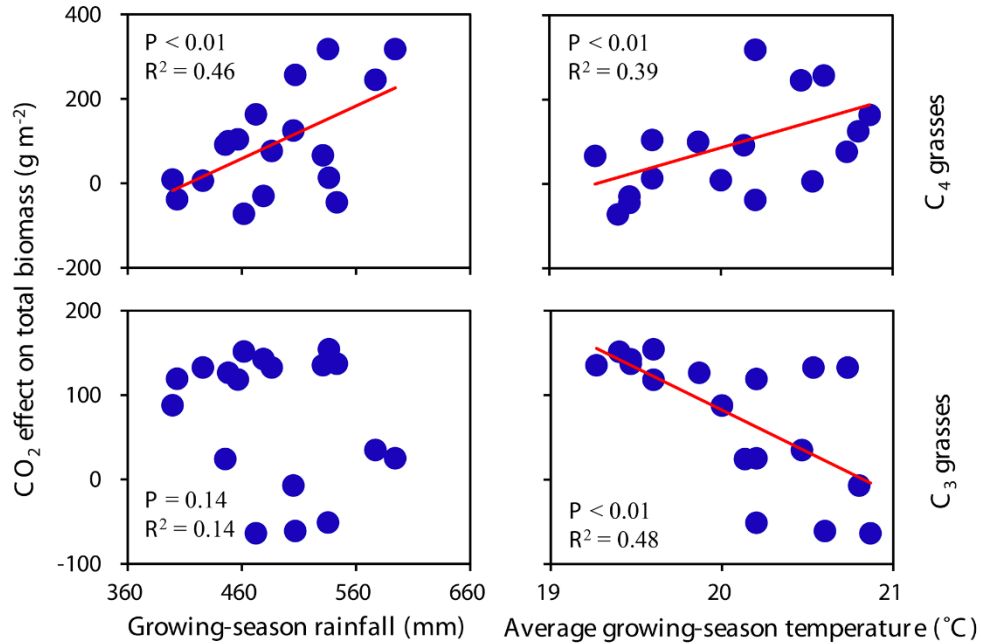
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**Fig. 1. Relationships between the CO<sub>2</sub> effect on total C<sub>4</sub>-C<sub>3</sub> biomass and growing-season climate.** The biomass data are from the measurements of Reich *et al.* (1). CO<sub>2</sub> effect size = biomass under eCO<sub>2</sub> condition – biomass under ambient CO<sub>2</sub> condition. The Cedar Creek weather data are from Fort Snelling near the Saint Paul International Airport ([www.wunderground.com/history/airport/KMSP](http://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<sub>2</sub> effect size and climatic factors were analyzed using generalized linear models (CO<sub>2</sub> effect size – temperature + rainfall; family = Gaussian; link = identity). The partial R<sup>2</sup> of each climatic factor was obtained using the *rsq*. partial function with the *rsq* package in the R version 3.2.2.

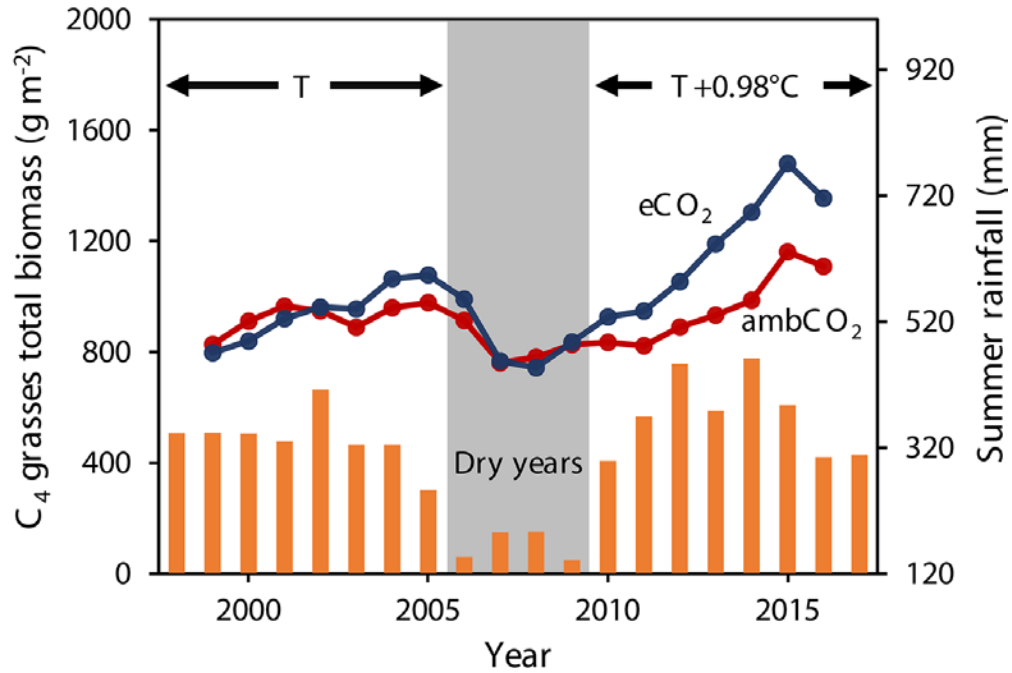


Fig. 2. Inter-annual trajectories of C<sub>4</sub> total biomass at the ambient (red) and elevated CO<sub>2</sub> (blue) levels and summer rainfall (orange). The Biomass data from the measurements of Reich *et al.* (1) are shown as 3-yr moving averages centered over the middle of each 3-yr group. The Cedar Creek weather data are from Fort Snelling near the Saint Paul International Airport.

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