

## A 23 m.y. record of low atmospheric CO<sub>2</sub>

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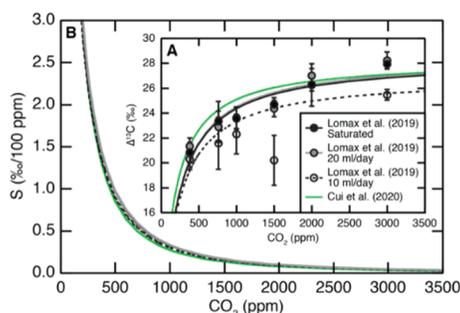
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Within their Comment, Jardine and Lomax (2021) object to our reconstruction of low atmospheric CO<sub>2</sub> across the last 23 m.y. (Cui et al., 2020) by claiming to identify three fatal problems in our analysis: First, that the predictive relationship we employ between carbon isotope value and CO<sub>2</sub> (Schubert and Jahren, 2012, 2015, 2018) is negated by the study of Lomax et al. (2019); second, that the widely observed effect of water stress on carbon isotope discrimination ( $\Delta^{13}\text{C}$ ) in land-plant derived carbon prohibits the use of  $\Delta^{13}\text{C}$  to infer CO<sub>2</sub>; third, that our method for CO<sub>2</sub> reconstruction acts to systematically underpredict CO<sub>2</sub>. Below we address all three points.

To address the first point: to perform CO<sub>2</sub> reconstruction, we rely on plant growth experiments (Schubert and Jahren, 2012) that showed  $\Delta^{13}\text{C}$  value increasing with CO<sub>2</sub> according to a hyperbolic response described as the following:

$$\Delta^{13}\text{C} = [(A)(B)(\text{CO}_2 + C)] / [(A) + (B)(\text{CO}_2 + C)], \quad (1)$$

where A is the asymptote, B is a measure of responsiveness, and C offsets the y-intercept to 4.4‰ (Schubert and Jahren, 2012). Figure 1 and Table 1 show that the data produced by Lomax et al. (2019) actually confirm our original work, although this has not been recognized by the authors: all treatments performed are well-described by the hyperbolic relationship we apply (Fig. 1A), producing highly comparable values of A, B and C (Table 1).



**Figure 1.** The effect of CO<sub>2</sub> on  $\Delta^{13}\text{C}$  value. (A)  $\Delta^{13}\text{C}$  values from Lomax et al. (2019) and Cui et al. (2020) follow a hyperbolic relationship with increasing CO<sub>2</sub> (Equation 1), as previously described (Schubert and Jahren, 2012, 2015) and used within Cui et al. (2020). Curve fitting parameters excluding the outlier measured at 1500 ppm (10 ml/day) are listed in Table 1. (B) First derivative, or slope, of the hyperbolic relationships (S, Equation 2) is functionally identical for all treatments.

**Table 1.** Comparison of curve fitting parameters A, B, and C (Equation 1) and calculated S values (Equation 2) using experiments of Lomax et al. (2019) compared with Cui et al. (2020).

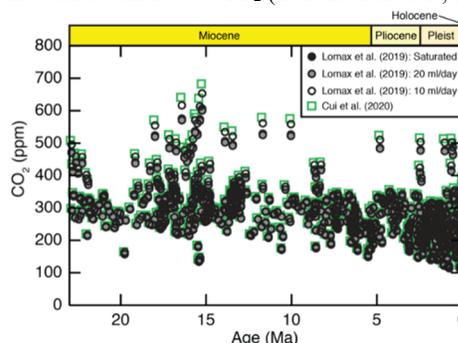
	A	B	C	F	S(‰/100 ppm)					
	ppm	ppm	ppm	ppm	380	760	1000	1500	2000	3000
Lomax et al. (2019): 10 ml/day	26.9	0.17	31.0	0.84	1.3	0.5	0.3	0.1	0.1	0.04
Lomax et al. (2019): 20 ml/day	28.5	0.17	30.5	0.82	1.4	0.5	0.3	0.2	0.1	0.05
Lomax et al. (2019): Saturated	28.3	0.17	30.6	0.90	1.4	0.5	0.3	0.2	0.1	0.05
Cui et al. (2020)	28.3	0.22	23.9	0.88	1.3	0.4	0.3	0.1	0.1	0.04

As for the second point: the most extreme water stress treatment (10 ml/day) within Lomax et al. (2019) yielded a value of A that was lowered by 1.4‰ compared to the A value resulting from our myriad experiments used within Cui et al. (2020), while the A value for all other treatments (including 20 ml/day) within Lomax et al. (2019) match our value of A to within 0.2‰ (Table 1). Lower values for A under water stress are fully consistent with our expectations (Schubert and Jahren, 2018), again confirming our earlier work. More importantly, S, the key value that we use to reconstruct CO<sub>2</sub> (defined as the change in  $\Delta^{13}\text{C}$  value per change in CO<sub>2</sub> and is calculated as the first derivative of Equation 1):

$$S = (A^2)(B) / [A + (B)(\text{CO}_2 + C)]^2, \quad (2)$$

is unaffected by the range of A values produced by all treatments within Lomax et al. (2019), including that of the most extreme water-stress

treatment (Fig. 1B and Table 1). We illustrate this by reconstructing CO<sub>2</sub> using our proxy with A, B, and C determined from the data set of Lomax et al. (2019) and compare it with CO<sub>2</sub> reconstructed within our original paper (Fig. 2). We find that the average difference between our original reconstruction and that determined using the 10 ml/day, 20 ml/day and saturated experiments of Lomax et al. (2019) is 1.0 ppm, 3.0 ppm and 2.7 ppm, respectively (Fig. 2), thus reinforcing our previous conclusion that the effect of CO<sub>2</sub> on  $\Delta^{13}\text{C}$  value is independent of water availability (Schubert and Jahren, 2018) and the choice of A has only a very small effect on reconstructed CO<sub>2</sub> (Cui and Schubert, 2016).



**Figure 2.** CO<sub>2</sub> calculated using our proxy with the experimental data of Lomax et al. (2019) compared to that of Cui et al. (2020). The average difference between our values and those produced using Lomax et al. (2019) = 2.2 ppm.

Third, the claim that our approach acts to systematically underpredict CO<sub>2</sub> is directly contradicted by a test that we performed years ago (i.e., Schubert and Jahren, 2015), wherein we compared the CO<sub>2</sub> record predicted by our method to CO<sub>2</sub> levels observed over the last 30,000 years via ice cores. The result was a CO<sub>2</sub> record that closely matched the ice cores ( $r^2 = 0.95$ ), and was not affected by calculating CO<sub>2</sub> based on changes in  $\delta^{13}\text{C}$  value (i.e.,  $\delta^{13}\text{C}_{\text{anomaly}}$ ; Cui et al. 2020) or changes in  $\Delta^{13}\text{C}$  value (i.e.,  $\Delta(\Delta^{13}\text{C})$ ; Schubert and Jahren, 2015) (average difference = 0.2 ppm). Moreover, the uncertainty analysis used in Cui and Schubert (2016) is very conservative: the 95% confidence interval allows for a 6.4‰ range in  $\Delta^{13}\text{C}$  value, which is comparable to the range of plant-tissue  $\Delta^{13}\text{C}$  values spanning xeric to rainforest ecosystems, and is ~5× larger than the range of plant-tissue  $\Delta^{13}\text{C}$  values measured within the water treatment experiments of Lomax et al. (2019).

In summary, we stand by our original analyses and results with no reservations. We have deposited all associated data, with the 95% confidence intervals, in the paleo-CO<sub>2</sub>.org database.

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