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Quantifying the influence of water stress on the use of plant isotopes to estimate paleo-CO_2

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Our previous work demonstrated a hyperbolic relationship between CO_2 concentration and net carbon isotope discrimination ($\Delta^{13}C$) in C_3 land plants. We ascribed the mechanism to photorespiration, a conserved trait through the history of photosynthesis. From the first, our goal was to use the $\Delta^{13}C$ value of fossilized plant material preserved as terrestrial organic matter (TOM) as a proxy for paleo-CO_2. The most stringent critique of this method was that it ignores the potential effect of water stress on $\Delta^{13}C$, an effect that, if present, would lead to a systematic underestimate of CO_2. Here, we report new experimental data comparing the magnitude of $\Delta^{13}C$ response for plant tissue grown under the simultaneous modulation of water stress and CO_2. Within this work, we germinated a total of 164 *Arabidopsis thaliana* seeds within individual pots inside five controlled growth chambers at CO_2 ranging from 389 to 2175 ppmv, and three levels of water stress: field capacity (FC, θ_m = 1.50 g g^{-1} or 60%), permanent wilting point (PWP, θ_m = 0.44 g g^{-1} or 30%), and a midpoint level that represented substantial, but not terminal, water stress (θ_m = 0.83 g g^{-1} or 45%).

We measured an increase in $\Delta^{13}C$ value with increasing CO_2 at each level of water stress. The response was indistinguishable comparing the plants grown at θ_m = 60% and 45%, and only differed for plants growing at the minimum hydration required to prevent death. Yet, the same increase in $\Delta^{13}C$ value with increasing CO_2 occurred at all levels of water stress, including both PWP and FC, as well as at the midpoint, θ_m = 45%. Thus we expect that the general relationship between $\Delta^{13}C$ and CO_2 that we quantified previously holds true for all environmental conditions that support plant growth, and will present several applications and misapplications of using $\Delta^{13}C$ value to predict CO_2 across the Phanerozoic.