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Separating the effects of CO_2 and water stress on carbon isotope discrimination in C_3 land plants

Brian A. Schubert[1] and A. Hope Jahren[2]

[1]*School of Geosciences, University of Louisiana at Lafayette, Lafayette, LA 70504*
[2]*Centre for Earth Evolution and Dynamics, University of Oslo, N-0315, Oslo, Norway*

The relative importance of carbon dioxide concentration *versus* water availability on the net carbon isotope discrimination ($\Delta^{13}C$) between that of plant tissue ($\delta^{13}C$) and that of the atmosphere ($\delta^{13}C_{atm}$) has been debated. We have previously quantified CO_2-dependency using the model plant *Arabidopsis thaliana* grown across CO_2 levels ranging from sub- to super-ambient (i.e., 97 – 2255 ppm), and ascribed the mechanism to photorespiration, a conserved trait through the history of photosynthesis. Within these experiments, each plant was grown under conditions of luxury nutrient- and water-availability. Here, we report on a suite of experiments designed to separate and quantify the isotopic dependency of plant tissue $\delta^{13}C$ upon both CO_2 and soil moisture content (θ_m).

The experimental application of a consistent and quantifiable level of water stress across an extended period is difficult to achieve; we designed our experiments after the methods of Granier et al. (2005), which involves first determining the soil's water retention capacity in units of g water g^{-1} dry soil (reported in terms of percent), then adjusting this value to determine the soil moisture content below retention capacity, at which plants fail to grow. For our experiments with *A. thaliana*, water retention capacity was 60%, and failure to grow due to insufficient moisture occurred at 27%. To these we added levels of 30% and 45% in order to capture the full range of water stress that plants can endure. At each of the four levels of soil moisture, we grew 12 plants to maturity under ambient, 685, 1076, 1583, and 2175 ppm CO_2 (the full range of CO_2 levels estimated across the last 420 million years of Earth history).

Within our experiments, $\Delta^{13}C$ value increased with increasing CO_2 across all water treatments. We found changes in $\Delta^{13}C$ value were greatest at low CO_2 and diminished with increasing CO_2. We conclude that the effect of CO_2 on $\Delta^{13}C$ value does not change across water treatments; $\Delta^{13}C$ values increased with increasing θ_m or increasing CO_2, as expected. These experiments demonstrate that both CO_2 and soil moisture affect $\Delta^{13}C$ value, but that these effects are quantitatively independent of each other, i.e. the soil moisture effect is not influenced by the CO_2 level and vice versa. We conclude that the effect of CO_2 on $\Delta^{13}C$ value is the same for both wet and dry environments.