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In situ estimation of hydrogen isotope fractionation associated with sucrose and cellulose synthesis from leaves to roots

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Plant cellulose hydrogen (H) stable isotope compositions (δ^2H) integrate hydrological and biochemical information, and therefore measurements from archives such as tree rings can be valuable for understanding past climate and plant metabolic responses to environmental change. Although the hydrological component that is integrated into cellulose δ^2H values is relatively well understood, the biochemical reactions that can alter δ^2H values of metabolites used for cellulose biosynthesis remain cryptic. Attempts at establishing models to simplify the interpretation of cellulose δ^2H values have been made, like the widely used cellulose δ^2H model by Roden et al. (2000) using the terms quantified by Yakir & DeNiro (1990). However, independent quantification of the parameters in this model, and assessment of their variability with respect to plant C metabolism, has been limited.

The cellulose δ^2H model uses the δ^2H compositions of leaf water and source water, autotrophic and heterotrophic ^2H-fractionation (ε_A and ε_H, respectively), and the proportion of carbon (C) bound H that exchanges with xylem water during cellulose biosynthesis (f) to explain variation in cellulose δ^2H values. By growing plants along a gradient of source water δ^2H values under autotrophic and heterotrophic conditions, the original, ε_A, ε_H, and f were determined for the aquatic plant *Lemna gibba* L.. One drawback of this approach is that it assumes these terms are the same when plants are grown in the light vs the dark. We recently reassessed the model for terrestrial plants by measuring δ^2H values of leaf sucrose and found species variation in ε_A (Holloway-Phillips et al., 2022), but were unable to resolve variation associated with f and ε_H.

In the present experiment we assessed a new experimental approach to quantify all model parameters for autotrophically grown plants using regression analysis. This required growing plants with variation in the isotopic offset between xylem water and leaf water (Δ_{LW}) and measuring sucrose and cellulose δ^2H values from leaves and roots. In a previous study we determined that mutation-induced inhibition of starch synthesis in leaves resulted in higher cellulose δ^2H values compared with the wildtype, which was hypothesized to occur preceding sucrose synthesis in the leaves (Baan et al., 2023). Using this new approach, we tested whether this effect was indeed mostly established in source cells during de novo sucrose synthesis (ε_A), or was a result of ^2H-fractionating processes in sink cells prior to cellulose synthesis (ε_H and f).

Preliminary analyses show an increase in leaf sucrose δ^2H values in the mutant relative to the wild type, implying that ε_A is also dependent on plant C metabolism within a given species.