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Can hydrogen isotopes ratios in plants be used to inform the metabolic consequences of C allocation patterns

Ansgar Kahmen, Kerstin Treydte, and Meisha Holloway-Phillips

Basel, Basel, Switzerland (ansgar.kahmen@unibas.ch)

All hydrogen in plant compounds derives from plant water; however, fractionation and isotopic exchange with local tissue water theoretically occurs in the synthesis of sucrose, and during downstream metabolic steps before the eventual synthesis of cellulose in sink tissue. The net result is that the relationship between the hydrogen isotope composition (d^2H) of cellulose and plant water may be weak. Whilst isotopic exchange and biosynthetic fractionation complicate our ability to recover the hydroclimate signal, the potential for d^2H variation to provide information on plant metabolic responses to environment is gaining traction.

Not all the fractionation factors associated with metabolic reactions are known for hydrogen, and even if they were, the apparent isotopic effect is dependent on the flux through the pathway. However, some generalisations have been made: 1) triose phosphate from the Calvin cycle is proposed to have a d^2H lower than leaf water due to the transfer of hydrogen from NADPH to glyceraldehyde-3-phosphate being highly 2H -depleted; 2) as plant water is enriched compared with primary photosynthetic products, isotopic exchange with water will generally result in a 2H -enrichment of downstream sugars; and, 3) the more times sugars pass through fractionating reactions, the more enriched the resulting sugar pool has been hypothesised to become. This has led to two general hypotheses relating to plant C-use: 1) when sink demand for sugars is low compared with source availability, the residence time of sugars in sink cells may increase leading to greater isotopic exchange. In other words, cellulose would be relatively 2H -enriched under high vs under low source-to-sink ratio; and 2) long-term starch stores may be 2H -enriched relative to current assimilates, assuming starch undergoes greater isotopic exchange prior to being stored or reflecting on-going metabolic exchange between local sugar-starch pools in parenchyma cells. In other words, cellulose would be relatively 2H -enriched where the contribution of stored C compared to current assimilates to cellulose synthesis, is greater.

To test these ideas, we are currently investigating how d^2H varies inter-annually and vertically within mature trees of *Fagus sylvatica* and *Picea abies* collected at the Canopy Crane Site II, Hölstein, Switzerland. Assessment is initially being made in samples collected from the top and base of the main stem as well as in collar roots, for the years 2017, 2018 and 2019. Previous

studies have observed that under water-stress conditions, C allocation below the canopy can be reduced. The summer of 2018 was exceptionally hot and dry; thus, we anticipate that the inter-annual $\delta^2\text{H}$ patterns will contrast by vertical position, particularly in 2018 compared with 2017 and 2019. Relative changes in the vertical distribution of C will be determined through assessment of the tree-ring width and circumference. For a direct appraisal of hypothesis 2, we are currently developing analytical capacity to measure the $\delta^2\text{H}$ of plant extracted sucrose and starch.