This is the published version of a paper presented at *EGU General Assembly 2018, Vienna, Austria, April 8-13, 2018*.

Citation for the original published paper:


N.B. When citing this work, cite the original published paper.

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http://urn.kb.se/resolve?urn=urn:nbn:se:umu:diva-145982
Measurements of carbon isotope contents of plant organic matter provide important information in diverse fields such as plant breeding, ecology, biogeochemistry and paleoclimatology. They are currently based on $^{13}$C/$^{12}$C ratios of whole metabolites, but we show here that intramolecular ratios provide higher resolution information about long-term metabolic dynamics, and their environmental controls.

**Definitions:** Measurements were expressed in terms of intramolecular $^{13}$C discrimination, $\Delta_i$, where $i$ denotes individual C positions in tree-ring glucose ($\text{Fig. 1}$, solid line). In this notation a positive value denotes discrimination against $^{13}$C. Triose phosphate cycling (TPC) in tree-ring cells confounds leaf-level $^{13}$C signals by redistributing C between C-1 and C-6, C-2 and C-5, and C-3 and C-4. We described the process mechanistically, and used the model to remove the TPC effect from $\Delta_i$, yielding TPC-free intramolecular $^{13}$C discrimination, $\Delta_i'$. (See figure 1, dashed line).

**Question 1:** Is there intramolecular $^{13}$C variation in tree-ring glucose?

There are well-established differences in $^{13}$C abundances among intramolecular C positions in various metabolites, including glucose$^2$7. These differences are introduced by enzymatic reactions$^1$. They are not predictable based on current theory, and it is apparent from significant positional deviations between modelled and measured $^{13}$C pattern of plant hexoses from different tissues$^8$. We show here that intramolecular $^{13}$C patterns have important implications for biogeochemical applications, and are therefore important to measure.

**Answer:** Tree-ring glucose exhibits a pronounced non-random $^{13}$C pattern ($\text{Fig. 1}$). This is corroborated by measurements on 11 additional species, 6 angiosperm and 5 gymnosperm trees$^9$. Detected intramolecular $^{13}$C differences exceed 10‰ (solid line). Thus, they are an order of magnitude larger than intra-annual $^{13}$C variations of atmospheric CO$_2$. Moreover, their magnitude is similar to $^{13}$C differences reported for distinct plant metabolites$^{10}$, and to the whole C range reported for bulk plant materials, including C3 and C4 plants$^{11}$.

**Implications:** Wood cellulose (composed of glucose units) is one of the largest C pools, contributing to soil organic matter. Its turnover strongly impacts on the global C cycle. Isotopes are powerful tools for analysing soil C turnover. However, their use requires information about the isotopic composition of soil substrates, and their accuracy will benefit from the consideration of large intramolecular differences ($\text{Fig. 1}$). For instance, soil cellulose documentation and discrimination against soil conditions and plant activities (e.g. fractionation of plant and weathering) are important aspects of soil C turnover.

**Question 2:** Is the signal of Diffusion-Rubisco - DR - fractionation detectable at all C positions of tree-ring glucose?

DR fractionation refers to $^{13}$C fractionation by CO$_2$ diffusion from ambient air into plant chloroplasts and Rubisco-mediated CO$_2$ fixation (Farquhar mode)$^{12}$. Rubisco adds a single carbon from CO$_2$ to ribulose-1,5-bisphosphate. Therefore, DR fractionation cannot cause intramolecular $^{13}$C variation, i.e. it is not position-specific. If DR fractionation was the only temporary variable fractionation process in plants, its signal strength should be equal at all positional time series of $^{13}$C discrimination, $\Delta$. We tested this by analysing the linear relationships between $\Delta$ and air vapour pressure deficit (VPD), which we found to be the predominant control of DR fractionation at our site$^{13}$.

**Answer:** We found that VPD signal strengths vary among $\Delta_i$ ($\text{Fig. 2}$). The largest deviations from uniformity were detected in $\Delta_2$ and $\Delta_3$. While the slope of the $\Delta_{i=2}/\text{VPD}$ regression is significantly steeper than the slope of the $\Delta_{i=3}/\text{VPD}$ regression (ANOVA: p=0.02, n=2311), the slope of the $\Delta_{i=2}/\text{VPD}$ regression is not significantly different from zero (p=0.64). Thus, the VPD signal is stronger in $\Delta_2$ than in $\Delta_3$, and undetectable in $\Delta_1$.

**Implications:** The DR signal is retained in tree-ring glucose, to a position-specific manner. This suggests that PR fractionations influence $\Delta_i$, and have had varying effects on the 35-year long tree-ring series.

**Question 3:** Does tree-ring glucose record information about downstream metabolic processes?

Post Rubisco - PR - fractionation denotes $^{13}$C fractionation by enzymes acting downstream of Rubisco. This type of fractionation is known to occur at individual C positions within metabolites$^2$, i.e. it is position-specific. PR fractionation occurs at metabolic branch points$^3$. Theory predicts that the linear relationships between $\Delta$ and VPD air vapour pressure deficit (VPD), which we found to be the predominant control of DR fractionation at our site$^{13}$.

**Answer:** We screened for position-specific signals by hierarchical cluster analysis of $\Delta_i$, and found four clusters: $\Delta_i'$ and $\Delta_i''$, and $\Delta_i$ and $\Delta_i$ are uncorrelated (r=0.08, p=0.68, and r=0.11, p=0.71, respectively), the signals of the respective clusters are independent of each other. Multiple signals may require multiple fractionation mechanisms; thus, besides the DR mechanism other fractionation mechanisms, i.e. PR mechanisms must be active.

**Implications:** Intramolecular $^{13}$C abundances of tree-ring glucose contain information about the dynamics of both primary C fixation and the downstream carbohydrate metabolism, thereby, many of the fractionations are clearly not negligible (Fig. 3). This may explain why the sensitivity of whole-molecule $\Delta$ values in tree rings to ecophysiological parameters is highly variable$^{14}$, and why coefficients of determination (R²) obtained by modelling $\Delta$ rarely exceed 50%. While the mechanisms behind observed PR fractionation signals require further attention, intramolecular $^{13}$C ratios clearly offer more information than whole-molecule ratios. This will likely facilitate retrospective assessment of ecophysiological and environmental traits unrelated to the diffusion-Rubisco mechanism.

**Material and Methods:** We pooled dated tree-ring samples - 19 Pinus nigra trees, 2 cores each - from a dry site in the Vienna region, Austria. Accordingly, our data reflect properties of the tree species at the site rather than properties of individual trees. Then, we extracted the pooled glucose moieties by hydrolysis of wood, and measured intramolecular $^{13}$C abundances by Nuclear Magnetic Resonance Spectroscopy on a suitable glucose derivative according to published procedures$^{15}$. Additionally, we measured $^{13}$C values by IRMS on the same derivatives. Then, isotopic balance calculation gave time series of annually-resolved $^{13}$C/$^{12}$C ratios for each individual C position of glucose extending from 1611 to 1995.

**References:**