TREE-RING CELLULOSE EXHIBITS SEVERAL INTERANNUAL ^{13}C SIGNALS ON THE INTRAMOLECULAR LEVEL

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Measurements of carbon isotope contents of plant organic matter provide important information in diverse fields such as plant breeding, ecophysiology, 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.

More Information →

$$\Delta = \text{AVERAGE}\left[\; \Delta_4' \;\; \Delta_6' \;\; \Delta_5' \;\; \Delta_1' \;\; \Delta_3' \;\right]$$
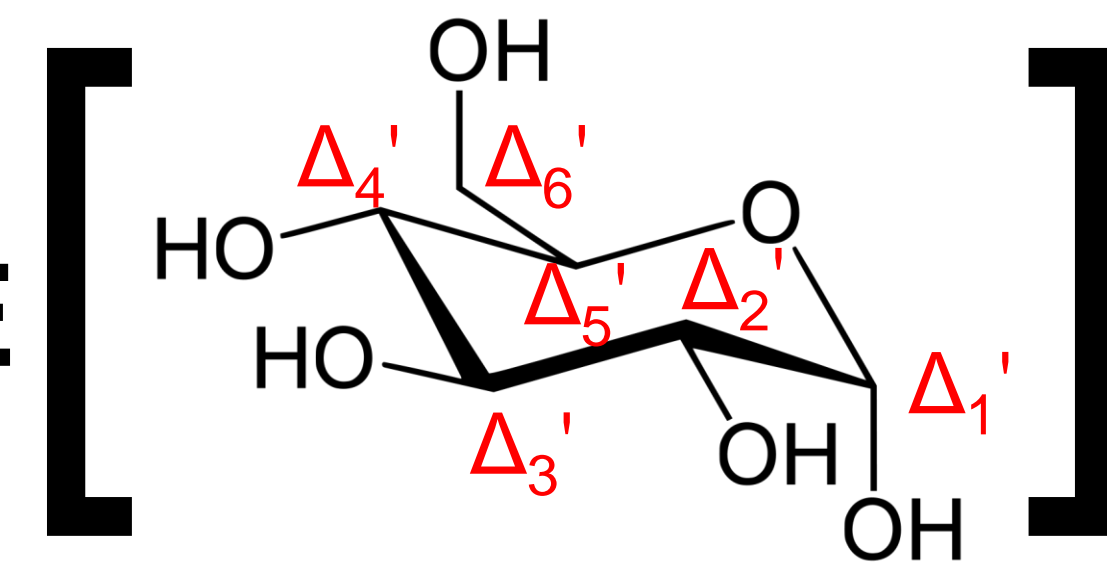
Definitions[2]: Measurements were expressed in terms of intramolecular ^{13}C discrimination, Δ_i, where i denotes individual C positions in tree-ring glucose (Fig. 1, solid line). In this notation a positive value denotes discrimination against ^{13}C. Triose phosphate cycling (TPC) in tree-ring cells 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 Δ_i, yielding TPC-free intramolecular ^{13}C discrimination, Δ_i' (Fig. 1, dashed line).

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 pools' 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[1]. Additionally, we measured δ^{13}C values by IRMS on the same derivatives. Then, isotope balance calculation gave time series of annually-resolved ^{13}C/^{12}C ratios for each individual C-H position of glucose extending from[1] 1961 to 1995.

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[3-7]. These differences are introduced by enzymatic reactions[5]. They are not predictable based on current theory, as 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[15], and are therefore important to measure.

Fig. 1

Δ_i and Δ_i' ±SE [‰] plotted against i = 1, 2, 3, 4, 5, 6
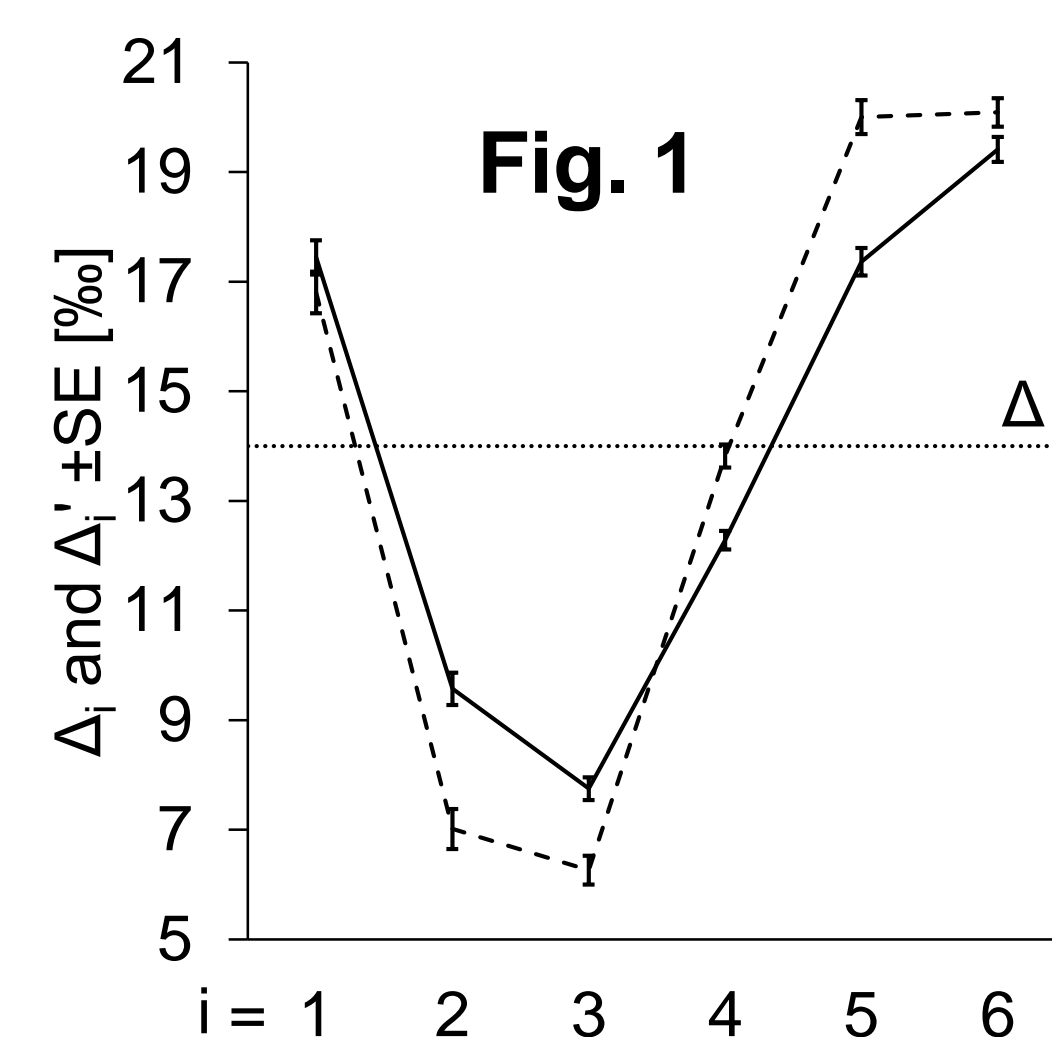
Answer: Tree-ring glucose exhibits a pronounced non-random ^{13}C pattern (Fig. 1). This is corroborated by measurements on 11 additional species, 6 angiosperm and 5 gymnosperm trees[2]. Detected intramolecular ^{13}C differences exceed 10‰ (solid line). Thus, they are an order of magnitude larger than intra-annual ^{13}C variations of atmospheric CO_2[9]. Moreover, their magnitude is similar to ^{13}C differences reported for distinct plant metabolites[10], and to the whole ^{13}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 global 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 (Fig. 1). For instance, soil cellulose decomposition occurs under both aerobic and anaerobic conditions via different metabolic pathways[12]. Because of the non-random ^{13}C distribution of wood glucose, different breakdown pathways will liberate CO_2 with distinct ^{13}C/^{12}C fingerprints. The ^{13}C/^{12}C of liberated CO_2 will equal the ^{13}C/^{12}C of substrate glucose, if glucose molecules are completely respired. If glucose is fermented (liberating C-3 and C-4), CO_2 with substantially more positive ^{13}C/^{12}C values will be released (see Definitions). Thus, considering positional ^{13}C differences in soil organic matter will enable better characterisation of C turnover pathways and quantification of heterotrophic soil respiration. This, in turn, will help reduce uncertainties in earth system models[15].

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 model)[16]. 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 temporally variable fractionation process in plants, its signal strength should be equal at all positional time series of ^{13}C discrimination, Δ_i'. We tested this by analysing the linear relationships between Δ_i' and air vapour pressure deficit (VPD), which we found to be the predominant control of DR fractionation at our site[2].

Fig. 2

Δ [‰] and Δ_i' [‰] versus Growing season air VPD [Pa]
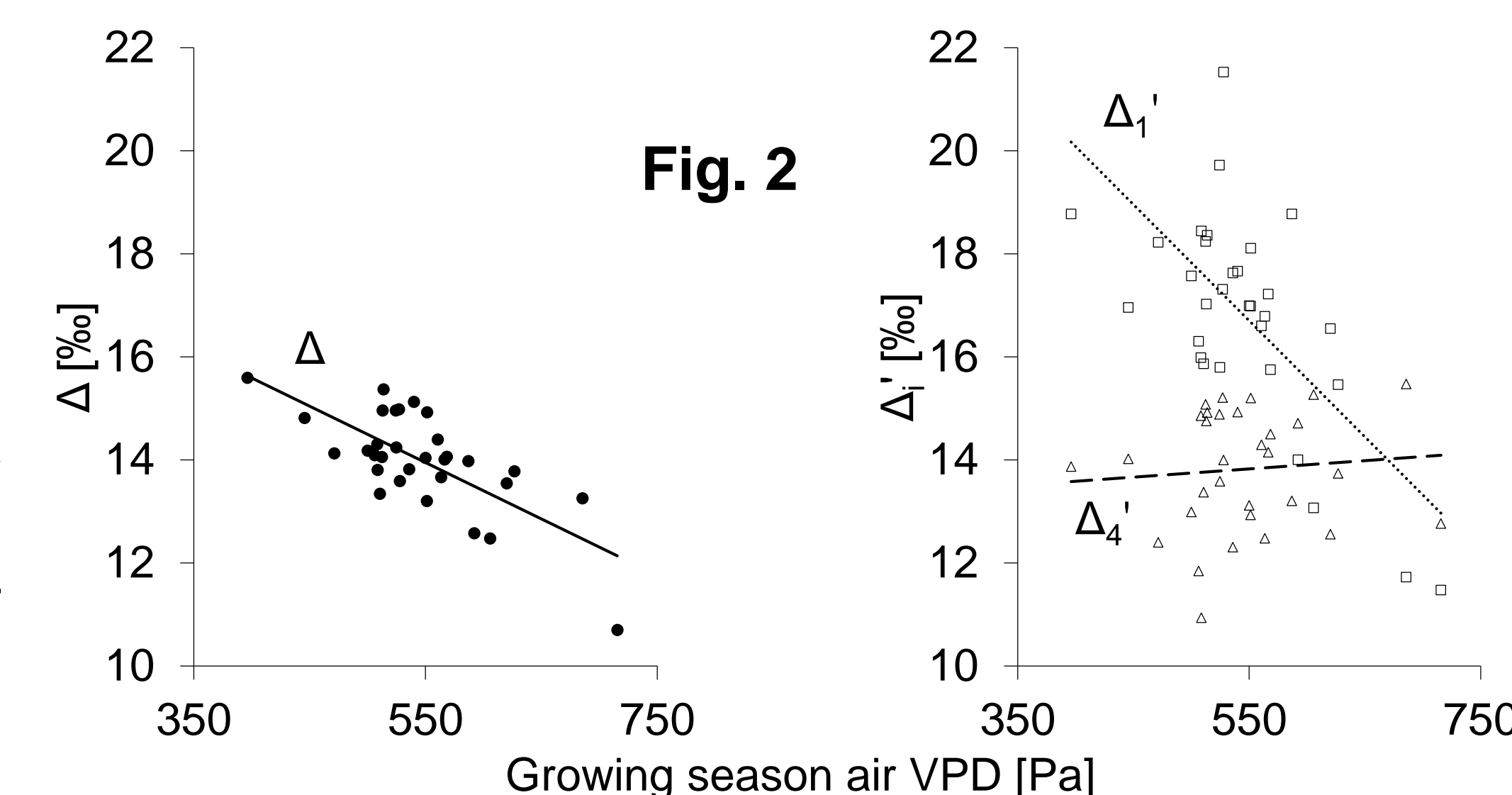
Answer: We found that VPD signal strengths vary among Δ_i' (Fig. 2). The largest deviations from uniformity were detected in Δ_1' and Δ_4'. While the slope of the Δ_1'~VPD regression is significantly steeper than the slope of the Δ~VPD regression (ANCOVA: p=0.02, n=2*31), the slope of the Δ_4'~VPD regression is not significantly different from zero (p=0.64). Thus, the VPD signal is stronger in Δ_1' than in Δ, and undetectable in Δ_4'.

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

References: [1] Chaintreau et al., Anal. Chim. Acta 788, 108-113 (2013). [2] Wieloch et al., Sci. Rep. 8, 5048 (2018). [3] Abelson & Hoering, PNAS 47, 623-632 (1961). [4] DeNiro & Epstein, Science 197, 261-263 (1977). [5] Gleixner & Schmidt, JBC 272, 5382-5387 (1997). [6] Gilbert et al. Nat. Prod. Rep. 29, 476-486 (2012). [7] Schmidt et al., Isotopes Environ. Health Stud. 51, 155-199 (2015). [8] Gilbert et al. PNAS 109, 18204-18209 (2012). [9] Levin et al., Tellus B 47, 23-34 (1995). [10] Gleixner et al., Planta 207, 241-245 (1998). [11] O'Leary, Phytochemistry 20, 553-567 (1981). [12] de Boer et al., FEMS Microbiol. Rev. 29, 795-811 (2005). [15] Flato, Wiley Interdiscip. Rev. Clim. Change 2, 783-800 (2011). [16] Farquhar et al., Aust. J. Plant Physiol. 9, 121-137 (1982). [17] Barbour et al., Tree Physiol. 34, 792-795 (2014).

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[7], i.e. it is position-specific. PR fractionation occurs at metabolic branch points[7]. Theory predicts that events such as changes in metabolite allocation at an isotope-sensitive branch point will change the intramolecular ^{13}C pattern. We tested whether intramolecular ^{13}C distributions carry signals reflecting such shifts.

Fig. 3

Euclidean distances (4–11): clusters Δ_3', * , Δ_1' ** Δ_2', Δ_4', Δ_5' *** Δ_6'
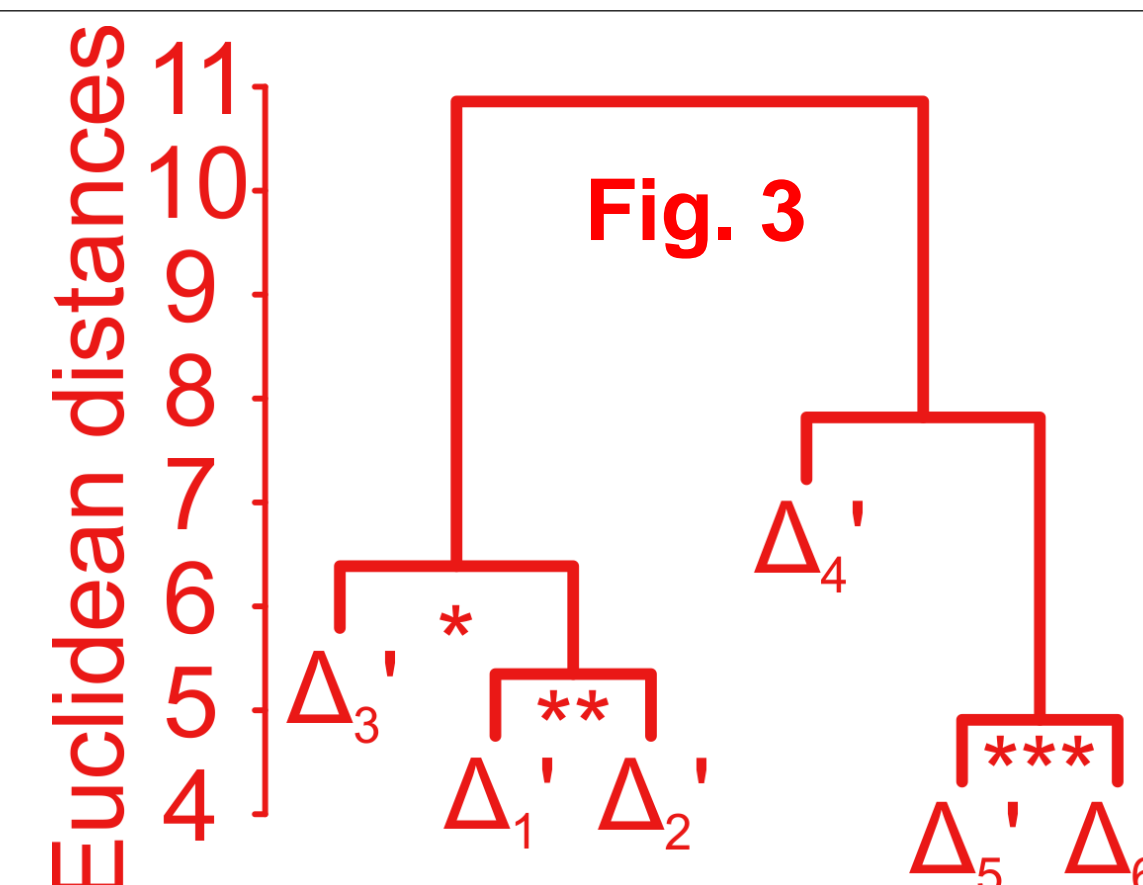
Answer: We screened for position-specific signals by hierarchical cluster analysis of Δ_i', and **found four clusters: Δ_1' + Δ_2', Δ_3', Δ_4', and Δ_5' + Δ_6' (Fig. 3).** Cluster formation and separation occur due to common and distinct variability, respectively. For instance, Δ_1' and Δ_2' as well as Δ_5' and Δ_6' share significantly correlated common signals (r=0.54, p=1.65*10^{-3}, and r=0.61, p=2.36*10^{-4}, respectively, n=31). As Δ_1' and Δ_6' as well as Δ_2' and Δ_5' are uncorrelated (r=0.08, p=0.68, and r=0.11, p=0.71, respectively, n=31), the signals of the respective clusters are independent of each other. Multiple signals 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 CO_2 fixation and the downstream carbohydrate metabolism. While DR fractionation explains much of the interannual variability of Δ, PR fractionations are clearly not negligible (Fig. 3). This may explain why the sensitivity of whole-molecule Δ values in tree rings to ecophysiological parameters is highly variable[17], and why coefficients of determination (R^2) obtained by modelling Δ 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.

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