

Supporting Information for

Shining a new light on the classical concepts of carbon-isotope dendrochronology

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Supporting Information Text

T1. Methods

Previously, we modelled Δ_i' as function of environmental and physiological variables as

$$\Delta_i' = \alpha + \beta_1 VPD + \beta_2 \varepsilon_{met} + \beta_3 RAD + \beta_4 TMP + e \quad (S1)$$

where α , β , and e denote the model intercept, coefficients, and error, respectively, and VPD , ε_{met} , RAD , and TMP denote March to November air vapour pressure deficit, average hydrogen isotope fractionation caused by metabolic processes at tree-ring glucose H¹ and H², April to September global radiation, and March to October air temperature, respectively (1). Based on these Δ_i' models, each Δ_i' series was here deconvoluted into its variable components yielding $\Delta_i' \sim VPD$, $\Delta_i' \sim \varepsilon_{met}$, $\Delta_i' \sim RAD$, $\Delta_i' \sim TMP$, and $\Delta_i' \sim e$ series. Subsequently, the percent contribution of each component to Δ_{glu} variability was calculated as

$$C_{\Delta_i' \sim x} = \frac{\text{Cov}(\Delta_i' \sim x, \Delta_{glu}) / 6}{\text{Var}(\Delta_{glu})} * 100 \quad (S2)$$

where Cov denotes the covariance between two series, Var denotes the variance of a series, and $x = \{VPD, \varepsilon_{met}, RAD, TMP, e\}$.

T2. Model residuals

Previously, Δ_i' was modelled as function of environmental and physiological parameters (Tables 1 and S1) (1). On average, Δ_i' data of the late period exhibit a standard error of $\pm 0.86\%$. Hence, not all of the variation in the Δ_i' series can be captured by modelling. Based on published procedures (2), the variance in Δ_i' series caused by measurement error was estimated and compared with the variance of Δ_i' model residuals (Table S2). For Δ_1' , Δ_3' , Δ_4' , and Δ_{5-6}' , the estimated error variance is similar to the models' residual variance indicating that the former largely accounts for the latter. By contrast, the residual variance of the Δ_2' model is about 3.5 times larger than the estimated error variance. Hence, the current Δ_2' model may not capture all of the systematic variance in Δ_2' , i.e., model extensions may be required.

T3. Contribution of carbon isotope signals to Δ_{glu} variation during the early period

For the late period, Δ_1' , Δ_2' , and Δ_3' were modelled as function of ε_{met} and/or VPD (Tables 1 and S1, 1). These models do not work for the early period (1). By contrast, RAD (data available from 1964) and TMP were found to affect Δ_4' to Δ_6' over the entire study period.

On average, Δ_i' data of the early period (1964 to 1980) exhibit a standard error of $\pm 0.94\%$. Hence, not all of the variation in the Δ_i' series can be captured by modelling. Based on published

procedures (2), the variance in Δ_i' series caused by measurement error was estimated and compared with the variance of Δ_i' residuals (Table S3). Since Δ_1' , Δ_2' , and Δ_3' data of the early period were not modelled, their entire variance constitutes residual variance. For Δ_2' , Δ_4' , and Δ_{5-6}' , the estimated error variance is similar to the residual variance indicating that the former largely accounts for the latter. By contrast, the residual variance of Δ_1' and Δ_3' is about 2 times larger than the estimated error variance. Hence, Δ_1' and Δ_3' may contain systematic variance which could, in principle, be captured by modelling.

Fig. S2 shows percent contributions of Δ_i' isotope signals found by modelling (Tables 1 and S1) and residuals to Δ_{glu} variation for the much less dynamic early period (1964 to 1980, $n = 15$, Δ_{glu} variance = 0.36‰). The contribution of Δ_1' is negligible and, therefore, not considered further. About $\approx 50\%$ of the total Δ_3' variance may be systematic variance (Table S3). If this variation results from DR discrimination, then DR discrimination accounts for $\approx 7.5\%$ of the total variance of Δ_{glu} while PR discrimination accounts for $\approx 43.6\%$.

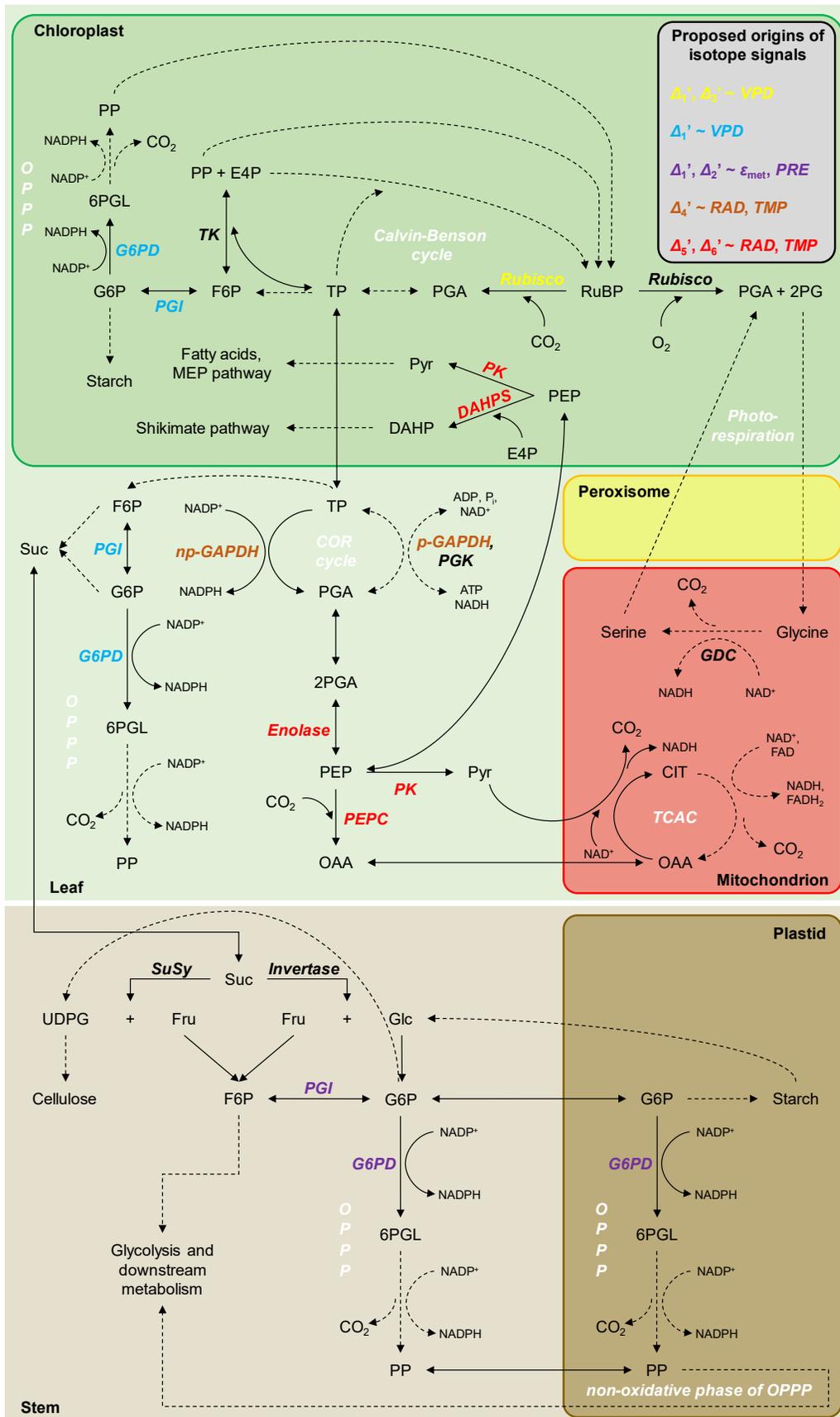


Fig. S1. Proposed metabolic origins of carbon isotope signals in tree-ring glucose. Modified figure from a previously published article (1). Dashed arrows indicate that intermediate reactions are not shown. Abbreviations: 2PG, 2-phosphoglycolate; 2PGA, 2-phosphoglycerate; 6PGL, 6-phosphogluconolactone; ADP, adenosine diphosphate; ATP, adenosine triphosphate; CIT, citrate; COR cycle, cytosolic oxidation-reduction cycle; DAHP, 3-Deoxy-D-*arabino*-heptulosonate 7-phosphate; DAHPS, DAHP synthase; E4P, erythrose 4-phosphate; F6P, fructose 6-phosphate; FAD, flavin adenine dinucleotide; Fru, fructose; G6P, glucose 6-phosphate; G6PD, G6P dehydrogenase; GDC, glycine decarboxylase complex; Glc, glucose; MEP pathway, methylerythritol 4-phosphate pathway; NAD⁺, nicotinamide adenine dinucleotide; NADP⁺, nicotinamide adenine dinucleotide phosphate; np-GAPDH, non-phosphorylating glyceraldehyde-3-phosphate dehydrogenase; OAA, oxaloacetate; OPPP, oxidative pentose phosphate pathway; PEP, phospho*eno*pyruvate; PEPC, PEP carboxylase; p-GAPDH, phosphorylating glyceraldehyde-3-phosphate dehydrogenase; PGA, 3-phosphoglycerate; PGI, phosphoglucose isomerase; PGK, phosphoglycerate kinase; P_i, inorganic phosphate; PK, pyruvate kinase; PP, pentose phosphate; *PRE*, precipitation; Pyr, pyruvate; Rubisco, ribulose-1,5-bisphosphate carboxylase/oxygenase; RuBP, ribulose 1,5-bisphosphate; *RAD*, global radiation; Suc, sucrose; SuSy, sucrose synthase; TCAC, tricarboxylic acid cycle; TK, transketolase; *TMP*, air temperature; TP, triose phosphates (glyceraldehyde 3-phosphate, dihydroxyacetone phosphate); UDPG, uridine diphosphate glucose; *VPD*, air vapour pressure deficit; Δ_i' , intramolecular ¹³C discrimination where *i* denotes individual glucose carbon positions and the prime denotes data corrected for ¹³C signal redistribution by heterotrophic triose phosphate cycling; ϵ_{met} , metabolic hydrogen isotope fractionation at glucose H¹ and H².

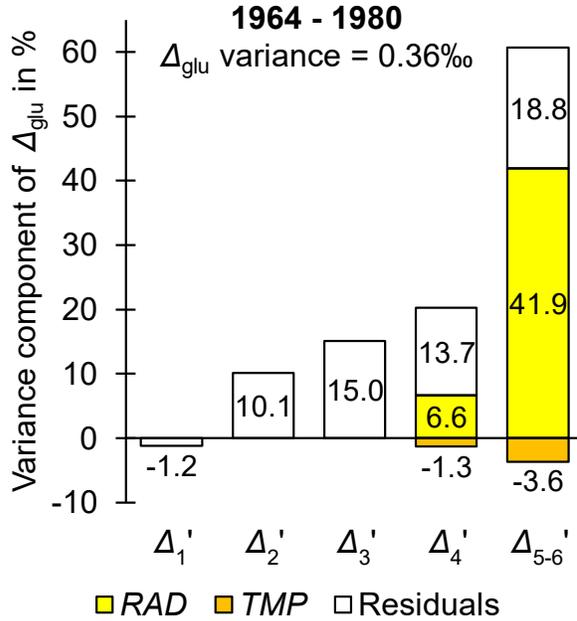


Fig. S2. Percent contributions of Δ_i' isotope signals and residuals to Δ_{glu} variation for the less variable early period (1964 to 1980, $n = 15$, Δ_{glu} variance = 0.36‰). Both Δ_4' and Δ_{5-6}' isotope signals and residuals were found by multiple regression modelling of Δ_i' as function of environmental covariates (16): *TMP*, March to October air temperature; *RAD*, April to September global radiation (data available from 1964). Since Δ_1' , Δ_2' , and Δ_3' data of the early period were not modelled, their entire variance constitutes residual variance. Δ_i' denotes intramolecular ^{13}C discrimination where i denotes individual glucose carbon positions and the prime denotes data corrected for ^{13}C signal redistribution by heterotrophic triose phosphate cycling. Δ_{glu} denotes whole-glucose ^{13}C discrimination. Glucose was extracted across an annually resolved tree-ring series of *Pinus nigra* from the Vienna Basin.

Table S1. Multiple linear regression models of Δ_i' as function of ε_{met} , March to November air vapour pressure deficit (*VPD*), March to July precipitation (*PRE*), April to September global radiation (*RAD*), and March to October air temperature (*TMP*).

$\Delta_1' \sim \varepsilon_{\text{met}} + \text{VPD}, 1983-1995$			
$R^2 = 0.87, \text{adj}R^2 = 0.84, p < 0.00004, n = 13$			
	Estimate	\pm SE	$p \leq$
Intercept	36.0	2.7	0.0000001
ε_{met}	-0.0187	0.0057	0.008
VPD	-0.0295	0.0047	0.0001
$\Delta_1' \sim \text{PRE} + \text{VPD}, 1983-1995$			
$R^2 = 0.82, \text{adj}R^2 = 0.79, p < 0.0002, n = 13$			
	Estimate	\pm SE	$p \leq$
Intercept	27.8	4.4	0.00008
PRE	0.0146	0.0061	0.04
VPD	-0.0280	0.0058	0.0007
$\Delta_2' \sim \varepsilon_{\text{met}}, 1983-1995$			
$R^2 = 0.54, \text{adj}R^2 = 0.50, p < 0.004, n = 13$			
	Estimate	\pm SE	$p \leq$
Intercept	11.8	1.7	0.00002
ε_{met}	-0.0351	0.0097	0.004
$\Delta_2' \sim \text{PRE}, 1983-1995$			
$R^2 = 0.43, \text{adj}R^2 = 0.37, p < 0.02, n = 13$			
	Estimate	\pm SE	$p \leq$
Intercept	-1.84	2.79	0.52
PRE	0.0274	0.0096	0.016
$\Delta_3' \sim \text{VPD}, 1983-1995$			
$R^2 = 0.57, \text{adj}R^2 = 0.53, p < 0.003, n = 13$			
	Estimate	\pm SE	$p \leq$
Intercept	14.3	2.2	0.000040
VPD	-0.0143	0.0037	0.0030
$\Delta_4' \sim \text{RAD} + \text{TMP}, 1964-1995$			
$R^2 = 0.15, \text{adj}R^2 = 0.09, p = 0.12, n = 28$			
	Estimate	\pm SE	$p \leq$
Intercept	8.33	4.73	0.09
RAD^(a)	-0.00266	0.00160	0.1
TMP	0.931	0.439	0.04
$\Delta_5' \sim \text{RAD} + \text{TMP}, 1964-1995$			
$R^2 = 0.66, \text{adj}R^2 = 0.64, p = 0.000001, n = 28$			
	Estimate	\pm SE	$p \leq$
Intercept	24.8	4.3	0.000005
RAD	-0.0103	0.0015	0.0000002
TMP	1.81	0.40	0.0001

(Table S1, continued on the next page)

(Table S1, continued)

$\Delta_6' \sim RAD + TMP, 1964-1995$			
$R^2 = 0.47, adjR^2 = 0.43, p = 0.0003, n = 28$			
	Estimate	$\pm SE$	$p \leq$
Intercept	27.3	4.2	0.0000009
<i>RAD</i>	-0.00658	0.00144	0.0001
<i>TMP</i>	0.876	0.393	0.04
$\Delta_{5-6}' \sim RAD + TMP, 1964-1995$			
$R^2 = 0.72, adjR^2 = 0.70, p = 0.0000001, n = 28$			
	Estimate	$\pm SE$	$p \leq$
Intercept	26.0	3.1	0.00000001
<i>RAD</i>	-0.00843	0.00105	0.00000002
<i>TMP</i>	1.35	0.29	0.00009

These models were published previously (1). ϵ_{met} , Δ_i' , and Δ_{5-6}' denote hydrogen isotope fractionation by metabolic processes at glucose H¹ and H², carbon isotope discrimination at glucose carbon position, *i*, and the arithmetic average of Δ_5' and Δ_6' , respectively. Glucose was extracted across an annually resolved tree-ring series of *Pinus nigra* from the Vienna Basin.

(a) Compared to other Δ_i' series, the systematic variance in Δ_4' is relatively low, which may explain the relatively low significance of its relationship with *RAD*.

Table S2. Estimated error variance in Δ_i' series and residual variance of Δ_i' models for the late period (1983 to 1995, $n = 13$).

	Δ_1'	Δ_2'	Δ_3'	Δ_4'	Δ_{5-6}'
Estimated error variance [‰]	0.71	0.73	0.71	0.77	0.47
Residual variance [‰]	0.78	2.67	0.57	1.00	0.35

Table S3. Estimated error variance in Δ_i' series and residual variance of Δ_i' for the early period (1964 to 1980, $n = 15$).

	Δ_1'	Δ_2'	Δ_3'	Δ_4'	Δ_{5-6}'
Estimated error variance [‰]	1.05	0.77	0.98	0.92	0.50
Residual variance [‰]	2.22	1.12	2.03	1.17	0.57

SI References

1. T. Wieloch, M. Holloway-Phillips, J. Yu, T. Niittylä, New insights into the mechanisms of post-rubisco isotope fractionation from combined analysis of intramolecular ^{13}C and deuterium abundances in *Pinus nigra* tree-ring glucose. *bioRxiv*, 2024.02.21.581384 (2024). Submitted to *New Phyt.* on the 12th of January 2024.
2. M. B. Nilsson, E. Dåbakk, T. Korsman, I. Renberg, Quantifying relationships between near-infrared reflectance spectra of lake sediments and water chemistry. *Environ. Sci. Technol.* **30**, 2586–2590 (1996).