

Intramolecular ^{13}C analysis of tree rings provides multiple plant ecophysiology signals covering decades - Supporting Information

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Site and samples - Additional information

Table S1 summarises spatial and temporal information for all samples included in this study. Sampling sites cover a wide range of ecological conditions. Important ecological features of the *Pinus nigra* sampling site include an "open canopy with low competition among trees", "shallow, very dry soils", and "limited or completely absent forest management disturbances"¹. Dominant trees with umbrella-shaped crowns indicating regular water scarcity were selected for sampling. Two 5-mm cores of each of 19 trees (approximate ages, 92-156 years) were taken at breast height. These samples have been used in several former studies¹⁻⁵, for which their annual rings were carefully dated by standard dendrochronological methods⁶. To preclude growth-related signals in our data, we focused the subsampling on tree rings formed during 1961 to 1995 when all trees had reached a comparably stable canopy position. Thus, isotopic shifts due to the contribution of soil-respired CO_2 or related mechanisms should be absent or negligible.

Table S1. Spatial and temporal information for all samples included in this study.

	Species	Site	Tree rings [years]	Lat. [°]	Long. [°]	Altitude [m AMSL]
Angiosperms	<i>Acer saccharum</i>	Wisconsin, USA	1997-2009	45.7N	89.5W	480
	<i>Fagus sylvatica</i>	Austria	2005-2011	47.15N	14.28E	840
	<i>Juglans regia</i>	Kyrgyzstan	1850-1859	41.78N	71.93E	1400
	<i>Liquidambar styraciflua</i>	Tennessee, USA	2000-2005	35.9N	84.33W	230
	<i>Quercus robur</i>	England	1989-1994	52.83N	0.5E	25
	<i>Shorea superba</i>	Borneo, Malaysia	1970-1980	5.02N	117.82E	170
Gymnosperms	<i>Juniperus zeravshanica</i>	Uzbekistan	1850-1859	39.62N	68.49E	2200
	<i>Phyllocladus aspleniifolius</i>	Average of three samples:				
		Tasmania, Australia	2005-2011	43.13S	146.692E	225
		Tasmania, Australia	1980-1989	42.21S	145.43E	250
		Tasmania, Australia	1990-1999	42.21S	145.43E	250
	<i>Picea abies</i>	Austria	1910-1919	47.18N	14.16E	1300
	<i>Pinus nigra</i>	Austria	1961-1995	48.13N	16.23E	350
	<i>Pinus ponderosa</i>	Arizona, USA	1850-1860	32.41N	110.72W	2423
<i>Thuja plicata</i>	Idaho, USA	1850-1859	47.19N	116.29W	1380	

^{13}C EA-IRMS and ^{13}C NMR Spectroscopy - Additional information

For Elemental Analysis - Isotope Ratio Mass Spectrometry (EA-IRMS) measurements we used a Flash EA 2000 coupled to a DeltaV system (both from Thermo Fisher Scientific Inc., Bremen, Germany). Repeated measurements ($n \geq 2$) confirmed that the equipment provided high accuracy and precision (long-term standard deviation of instrumental measurements: $\pm 0.15\%$ SD).

For quantitative ^{13}C NMR, samples of 1,2-O-isopropylidene- α -D-glucofuranose were dissolved in 160 μl deionised water and D-acetonitrile (CAS: 2206-26-0, filling height=35 mm). Either 5 or 6 mg of the relaxation agent $\text{Cr}(\text{acac})_3$ (CAS: 21679-31-2; 5 mg for > 80 mg samples, 6 mg for 20 to 80 mg samples) were added. Samples of 3,6-anhydro-1,2-O-isopropylidene- α -D-glucofuranose were dissolved in D-acetonitrile (filling height=35 mm), and 8 mg $\text{Cr}(\text{acac})_3$ was added. We determined longitudinal relaxation times (T_1) by inversion recovery experiments and adjusted the recycle delay to allow for complete relaxation ($\geq 10 T_1$) of all relevant ^{13}C nuclei. Quantitative ^{13}C NMR spectra were acquired at 298 K using a 90° pulse of 8.07 to 8.7 μs , following published procedures⁷.

By increasing the measurement time with decreasing sample amounts, we achieved signal-to-noise ratios (accumulated over all the spectra) of 900 to 1670 (average: 1270) per sample. Samples <20 mg were excluded from analysis, because too much measurement time would have been required for sufficient precision. All free induction decays were processed by the same operator and protocol: exponential apodization with a line broadening of 1 Hz; automatic phase correction plus subsequent manual phase correction if required; automatic baseline correction of the 10-130 ppm spectral range by a 3rd order polynomial. Lorentzian line shape fitting was applied for signal deconvolution. We calculated average areas under ^{13}C NMR signals, S_i , by averaging integrals over all replicate spectra.

Intramolecular ^{13}C distributions of tree-ring glucose

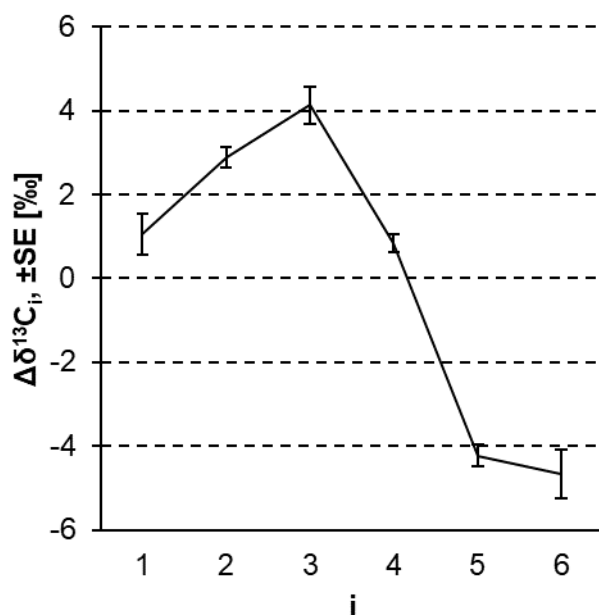


Figure S1. Intramolecular ^{13}C distributions of tree-ring glucose. Data were acquired for six angiosperm and six gymnosperm species from ecologically different sites with global coverage (Table S1). Solid line, average over all angiosperms and gymnosperms, $\pm\text{SE}$. In the biochemical literature, intramolecular ^{13}C distributions are commonly expressed as $\Delta\delta^{13}\text{C}_i$, the deviations of positional ^{13}C abundances from the molecular average. In contrast, in the ecophysiological literature the Δ scale representing fractionation against ^{13}C is preferred. To promote comparability with previously published intramolecular ^{13}C distributions in the biochemical literature, we here express ^{13}C distributions of tree-ring glucose in terms of $\Delta\delta^{13}\text{C}_i$. Consequently, the distributions here and in the main text (Fig. 1) appear vertically mirrored around the molecular average.

Test for autocorrelation in ^{13}C time-series

A tree ring formed in a particular year may have had significant input of stored glucose monomers from previous years. If so, ^{13}C time-series should exhibit autocorrelation signals. To test this possibility, we correlated each ^{13}C time-series with three temporally lagged versions of itself. No significant autocorrelation ($r=0.37$ to 0.40 for $p \leq 0.05$ with $n=28$ to 25) was detected (Tables S2, S3). Therefore, our analyses does not consider conditions of previous years.

Table S2. Pearson correlation coefficients of $\Delta_i(t)$ versus $\Delta_i(t+\tau)$ and $\Delta(t)$ versus $\Delta(t+\tau)$, with t and $(t+\tau)$ denoting ^{13}C time-series without and with temporal lag, respectively.

τ [years]	Δ_1	Δ_2	Δ_3	Δ_4	Δ_5	Δ_6	Δ
-1	0.22	0.29	0.10	-0.11	-0.23	0.25	0.07
-2	0.11	0.22	0.09	0.02	-0.01	-0.10	0.09
-3	-0.04	0.00	-0.30	-0.15	-0.09	-0.11	-0.19

Table S3. Pearson correlation coefficients of $\Delta'_i(t)$ versus $\Delta'_i(t+\tau)$, with t and $(t+\tau)$ denoting ^{13}C time-series without and with temporal lag, respectively.

τ [years]	Δ'_1	Δ'_2	Δ'_3	Δ'_4	Δ'_5	Δ'_6
-1	0.23	0.33	0.12	-0.10	-0.25	0.28
-2	0.07	0.26	0.07	-0.03	-0.01	-0.24
-3	-0.05	0.10	-0.27	-0.08	0.03	-0.18

Determination of growing season length

We define the growing season as the months with a median number of days ≥ 10 , over the observation period 1961-1995, with an average air temperature ≥ 5 °C. Figure S2 shows the number of days per month with ≥ 5 °C at Hohe Warte (Vienna, Austria, WMO ID: 1103500) during the period 1961-1995 ($n=35$). According to the applied definition, the growing season at our site extends from March to November.

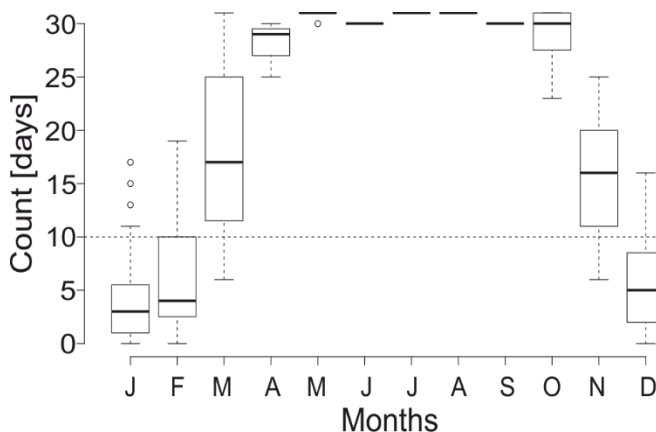


Figure S2. Number of days per month with average air temperatures ≥ 5 °C during the period 1961-1995 at the Hohe Warte meteorological station (Vienna, Austria, WMO ID: 1103500).

Effects of air vapour pressure deficit on ^{13}C discrimination

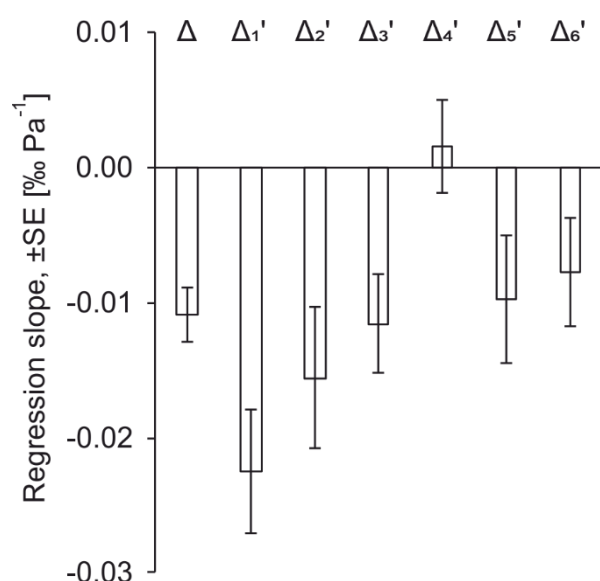


Figure S3. Effects of growing season air vapour pressure deficit (VPD) on whole-molecule ^{13}C discrimination, Δ , and on positional ^{13}C discrimination, Δ_i' . Data were acquired for tree-ring glucose of *Pinus nigra* laid down from 1961 to 1995 at a moisture-limited site in the Vienna basin. Bars represent slopes of ordinary least squares regressions, b and $b_i' \pm \text{SE}$.

ANCOVA

Growing season air vapour pressure deficit (VPD) is significantly correlated with both Δ and Δ_1' ($r=-0.72$, $p=5.4 \times 10^{-6}$ and $r=-0.68$, $p=3 \times 10^{-5}$, respectively, $n=31$). However, the slope of the Δ ~VPD regression is only half as steep as the slope of the Δ_1' ~VPD regression (Fig. 2, $-0.011 \pm 0.002 \text{SE } \text{‰ Pa}^{-1}$ and $-0.023 \pm 0.005 \text{SE } \text{‰ Pa}^{-1}$, respectively). According to ANCOVA, this slope difference is statistically significant ($p=0.02$, Table S4).

Table S4. Summary of iso~VPD*method ANCOVA modelling output, with iso denoting combined Δ and Δ_1' data, VPD denoting growing season air vapour pressure deficit, and method as a categorical variable assigning iso data to either Δ or Δ_1' . Asterisks denote the significance of model terms (*, $p \leq 0.05$; ***, $p \leq 10^{-3}$). The interaction term vpd:method contributes significantly to the model ($p=0.02$), showing that responses of Δ and Δ_1' to VPD differ significantly.

	Sum of squares	Df	F value	Pr(>F)	Significance
vpd	67.315	1	45.24	8.45E-09	***
method	121.947	1	81.95	1.086E-12	***
vpd:method	8.092	1	5.44	0.0232	*

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