Supporting Information for

Intramolecular isotope analysis enables multivariate climate reconstructions

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Extended methods

Tree-ring samples discussed here have been described in previous studies (1–6). Two 5 mm cores were taken at breast height from each of 19 *Pinus nigra* Arnold at Bierhäuselberg (Vienna region, Austria, 48.13° N, 16.23° E, 350 m AMSL). This site is unmanaged, has an open canopy, and shallow soil (2). Sampling targeted dominant trees with umbrella-shaped crowns indicating frequent water deficit. Tree-rings were dated by standard dendrochronological methods (see 7). On average, ring width timeseries start in 1865 (range: 1840 to 1918). To preclude significant isotope shifts due to developmental processes or the contribution of soil-respired CO_2, isotope analysis begins in 1961. At that point, all trees had reached a stable position in the canopy.

Tree-ring isotope data analysed here have been described previously (6). Individual tree rings within the period 1961 to 1995 were separated with a scalpel under a binocular microscope. All material (wood of 19 trees, 2 cores per tree) of a given year was pooled. Hence, the data represent the species at the site rather than individual trees. Subsequently, annual samples were randomised and ground (Retsch MM400, Haan, Germany). Wood glucose was derivatised to 1,2-O-isopropylidene-α-D-glucofuranose following published procedures (8). Proton nuclear magnetic resonance spectroscopy (NMRS) was used to check sample purity was ≥ 99.9%.

Following published procedures (9), whole-molecule $\delta^{13}C$ of the glucose derivative was measured with a Delta V isotope ratio mass spectrometer coupled to a Flash 2000 elemental analyser (Thermo Fisher Scientific Inc., Bremen, Germany; ≥ 2 repetitions per sample, long-term SD of the instrument: ± 0.15‰). Furthermore, intramolecular ^{13}C molar equivalents were determined by ^{13}C NMRS following published procedures (10). For this purpose, the samples were dissolved in a mixture of 160 μl deionised water, $Cr(acac)_3$ (CAS: 21679-31-2, 5 mg for > 80 mg samples, 6 mg for 20 to 80 mg samples), and D-acetonitrile (CAS: 2206-26-0, NMRS tube filling level = 35 mm). Per sample, 30 spectra were recorded on a Bruker 400 MHz AVANCE III fitted with a 5 mm BBFO SmartProbe (Bruker BioSpin GmbH, Rheinstetten, Germany) at 298 K using a 90° pulse of 8.07 to 8.7 μs. Prior to recording, longitudinal relaxation time (T_1) was measured by inversion recovery experiments. Recycling delays were set to ≥ $10T_1$ enabling complete relaxation of all ^{13}C nuclei. Measurement time was increased with decreasing sample amount. Samples < 20 mg were discarded since high precision measurement of these samples would have taken too much time (1977, 1978, 1981, and 1982). Cumulated over all spectra of a sample, signal-to-noise ratios between 900 and 1670 (average = 1270) were achieved. Free induction decays were processed in TopSpin 3.1 (Bruker BioSpin GmbH, Rheinstetten, Germany). We applied a positive exponential window function (line broadening of 1 Hz), automatic phase correction and subsequent manual phase correction if required, and automatic baseline correction within the 10 to 130 ppm range using a 3rd order polynomial function. Signal deconvolution based on Lorentzian line shape fits returned relative areas under signals. Averaging the values of the 30 spectra per sample, average relative signal areas, S_i, were calculated with i = {C-1, …, C-6, C-q, C-Me1, C-Me2}. Following published procedures (11), S_i was corrected for ^{13}C satellite contribution yielding ^{13}C molar equivalents, $S_{i(c)}$.

Leaf sucrose is translocated to tree-ring cells and converted to hexose derivatives which serve as precursors of tree-ring glucose. 40 to 50% of these derivatives participate in triose phosphate cycling (TPC). Since the triose phosphates glyceraldehyde 3-phosphate (a precursor of glucose C-4 to C-6) and dihydroxyacetone phosphate (a precursor of glucose C-1 to C-3) are in equilibrium, TPC causes 20 to 25% net carbon exchange among symmetry-related hexose carbon positions (C-1 and C-6, C-2 and C-5, and C-3 and C-4). Consequently, TPC convolutes ^{13}C signals introduced at the leaf level. TPC-free ^{13}C molar equivalents, $S_{i(c)}'$, and TPC-free ^{13}C discrimination, Δ_i', were calculated following published procedures (6).

Publicly accessible monthly resolved data of precipitation, air temperature, global radiation, sunshine duration, and relative humidity were obtained for the climate station Hohe Warte (Central Institution for Meteorology and Geodynamics, Vienna, Austria, 48.23° N, 16.35° E, 198 m AMSL, WMO ID: 1103500, 12). Air vapour pressure deficits were calculated following published procedures (13). Publicly accessible monthly resolved data of the standardised precipitation-evapotranspiration index calculated for integrated periods of 1, 3, 6, 8, 12, 16, 24, 36, 48 months and soil moisture were obtained for 48.25° N, 16.25° E (14, 15). Data of global radiation are available from 1964. Data of all other climate parameters and indices are available from 1961. Horizontal distances between the site of tree-ring sampling and the selected grid point and climate station are < 15 km. Vertical offsets are small. Hence, climate data and site conditions can be expected to be in good agreement.

Based on air temperatures during the study period (1961 to 1995), the growing season at the site was estimated to extend from March to November (6). Conifers form tree-ring cells over the course of several months (16). Therefore, all statistical analyses exclusively consider periods comprising ≥ 4 growing season months. According to autocorrelation analyses, growth of the trees studied here has not been significantly affect by interannual carry-over of carbon (6). Therefore, our statistical analyses do not consider climate conditions of previous years.

After mean-centring and unit-variance scaling of Δ_i' timeseries, Hierarchical Cluster Analysis was done with the functions dist() and hclust() of the 'stats' package in R (17) choosing Euclidean distances and Ward's fusion criterion as inputs (18). Ten-fold cross-validation of bivariate and multivariate regression models was done with the functions cv.lm() and CVlm() of the 'DAAG' package in R, respectively (19). Pearson correlation and ordinary least squares regression analysis were respectively done with the functions cor() and lm() of the 'stats' package in R (17). Fractions of systematic variance in isotope timeseries were estimated according to published procedures (20).

SI References

1. S. Leal, T. M. Melvin, M. Grabner, R. Wimmer, K. R. Briffa, Tree-ring growth variability in the Austrian Alps: the influence of site, altitude, tree species and climate. *Boreas* **36**, 426–440 (2007).

2. S. Leal, D. Eamus, M. Grabner, R. Wimmer, P. Cherubini, Tree rings of *Pinus nigra* from the Vienna basin region (Austria) show evidence of change in climatic sensitivity in the late 20th century. *Can. J. For. Res.* **38**, 744–759 (2008).

3. G. Strumia, R. Wimmer, M. Grabner, Dendroclimatic sensitivity of *Pinus nigra* Arnold in Austria. *Dendrochronologia* **15**, 129–137 (1997).

4. G. Strumia, "Tree-ring based reconstruction of precipitation in Eastern Austria," University of Natural Resources and Life Sciences, Vienna. (1999).

5. R. Wimmer, G. Strumia, F. Holawe, Use of false rings in Austrian pine to reconstruct early growing season precipitation. *Can. J. For. Res.* **30**, 1691–1697 (2000).

6. T. Wieloch, *et al.*, Intramolecular ^{13}C analysis of tree rings provides multiple plant ecophysiology signals covering decades. *Sci. Rep.* **8**, 5048 (2018).

7. J. H. Speer, *Fundamentals of tree-ring research* (The University of Arizona Press, 2010).

8. T. R. Betson, A. Augusti, J. Schleucher, Quantification of deuterium isotopomers of tree-ring cellulose using Nuclear Magnetic Resonance. *Anal. Chem.* **78**, 8406–8411 (2006).

9. R. A. Werner, B. A. Bruch, W. A. Brand, ConFlo III - An interface for high precision $\delta^{13}C$ and $\delta^{15}N$ analysis with an extended dynamic range. *Rapid Commun. Mass Spectrom.* **13**, 1237–1241 (1999).

10. A. Chaintreau, *et al.*, Site-specific ^{13}C content by quantitative isotopic ^{13}C Nuclear Magnetic Resonance spectrometry: A pilot inter-laboratory study. *Anal. Chim. Acta* **788**, 108–113 (2013).

11. B.-L. Zhang, M. Trierweiler, C. Jouitteau, G. J. Martin, Consistency of NMR and mass spectrometry determinations of natural-abundance site-specific carbon isotope ratios. The case of glycerol. *Anal. Chem.* **71**, 2301–2306 (1999).

12. A. M. G. Klein Tank, *et al.*, Daily dataset of 20th-century surface air temperature and precipitation series for the European Climate Assessment. *Int. J. Climatol.* **22**, 1441–1453 (2002).

13. W. Abtew, A. M. Melesse, "Chapter 5 - Vapor pressure calculation methods" in *Evaporation and Evapotranspiration*, (Springer Verlag, 2013), pp. 53–62.

14. S. M. Vicente-Serrano, S. Beguería, J. I. López-Moreno, A multiscalar drought index sensitive to global warming: The standardized precipitation evapotranspiration index. *J. Clim.* **23**, 1696–1718 (2010).

15. Y. Fan, H. van den Dool, Climate Prediction Center global monthly soil moisture data set at 0.5° resolution for 1948 to present. *J. Geophys. Res. Atmos.* **109** (2004).

16. H. E. Cuny, C. B. K. Rathgeber, D. Frank, P. Fonti, M. Fournier, Kinetics of tracheid development explain conifer tree-ring structure. *New Phytol.* **203**, 1231–1241 (2014).

17. R Core Team, R: A language and environment for statistical computing (2021).

18. J. H. Ward, Hierarchical grouping to optimize an objective function. *J. Am. Stat. Assoc.* **58**, 236–244 (1963).

19. J. H. Maindonald, W. J. Braun, Package ‘DAAG’ - Data analysis and graphics data and functions (2022).

20. 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).

List of Supplemental Datasets

Dataset 1: Coefficients of correlation of isotope-climate relationships.

Dataset 2: Coefficients of correlation of isotope-climate relationships.

Datasets for

Intramolecular isotope analysis enables multivariate climate reconstructions

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Supplemental Datasets

Dataset 1. Coefficients of correlation of isotope-climate relationships.

	VPD ~		*PRE ~*		*TMP ~*		*SM ~*		*SD ~*		*RAD ~*	
	$\Delta_{1\text{-}3}'$	$\Delta_{5\text{-}6}'$	$\Delta_{1\text{-}3}'$	$\Delta_{5\text{-}6}'$	$\Delta_{1\text{-}3}'$	$\Delta_{5\text{-}6}'$	$\Delta_{1\text{-}3}'$	$\Delta_{5\text{-}6}'$	$\Delta_{1\text{-}3}'$	$\Delta_{5\text{-}6}'$	$\Delta_{1\text{-}3}'$	$\Delta_{5\text{-}6}'$
MAMJ	-0.67	-0.27	0.46	0.21	-0.37	-0.09	0.23	0.31	-0.14	-0.34	-0.35	-0.48
MAMJJ	-0.64	-0.38	0.50	0.34	-0.32	-0.16	0.25	0.34	-0.12	-0.42	-0.33	-0.54
MAMJJA	-0.70	-0.40	0.50	0.29	-0.45	-0.21	0.23	0.35	-0.24	-0.47	-0.40	-0.59
MAMJJAS	-0.72	-0.40	0.52	0.24	-0.43	-0.20	0.25	0.38	-0.30	-0.45	-0.45	-0.61
MAMJJASO	-0.72	-0.38	0.51	0.22	-0.43	-0.17	0.25	0.34	-0.26	-0.39	-0.43	-0.60
MAMJJASON	-0.70	-0.39	0.50	0.16	-0.39	-0.24	0.21	0.36	-0.25	-0.38	-0.40	-0.60
AMJJ	-0.61	-0.51	0.51	0.47	-0.34	-0.44	0.25	0.37	-0.09	-0.54	-0.30	-0.65
AMJJA	-0.69	-0.49	0.50	0.41	-0.51	-0.46	0.23	0.39	-0.22	-0.58	-0.38	-0.69
AMJJAS	-0.70	-0.48	0.52	0.36	-0.48	-0.41	0.25	0.40	-0.28	-0.53	-0.42	-0.69
AMJJASO	-0.70	-0.45	0.51	0.35	-0.46	-0.36	0.25	0.36	-0.23	-0.46	-0.39	-0.67
AMJJASON	-0.69	-0.46	0.51	0.31	-0.40	-0.43	0.21	0.38	-0.22	-0.45	-0.37	-0.68
MJJA	-0.64	-0.49	0.45	0.35	-0.47	-0.52	0.19	0.38	-0.24	-0.60	-0.38	-0.67
MJJAS	-0.66	-0.48	0.49	0.31	-0.44	-0.47	0.23	0.40	-0.30	-0.54	-0.43	-0.67
MJJASO	-0.66	-0.46	0.47	0.29	-0.44	-0.42	0.22	0.35	-0.24	-0.45	-0.39	-0.64
MJJASON	-0.65	-0.46	0.47	0.23	-0.37	-0.49	0.19	0.37	-0.23	-0.43	-0.36	-0.64
JJAS	-0.64	-0.44	0.33	0.18	-0.35	-0.39	0.23	0.37	-0.26	-0.40	-0.37	-0.58
JJASO	-0.65	-0.42	0.27	0.12	-0.36	-0.34	0.22	0.31	-0.19	-0.30	-0.33	-0.53
JJASON	-0.63	-0.43	0.24	0.05	-0.27	-0.42	0.18	0.34	-0.18	-0.28	-0.29	-0.54
JASO	-0.60	-0.36	0.11	0.03	-0.35	-0.20	0.24	0.33	-0.25	-0.26	-0.33	-0.45
JASON	-0.58	-0.37	0.09	-0.05	-0.28	-0.31	0.19	0.37	-0.23	-0.23	-0.29	-0.46
ASON	-0.60	-0.30	-0.06	-0.34	-0.34	-0.27	0.15	0.34	-0.27	-0.07	-0.30	-0.36

Climate parameters: *PRE*, precipitation; *RAD*, global radiation; *SD*, sunshine duration; *SM*, soil moisture; *TMP*, air temperature; *VPD*, air vapour pressure deficit. Climate data were averaged for all ≥ 4-month periods of the growing season (March to November). Months were abbreviated by their initial letters. $\Delta_{1\text{-}3}$' and $\Delta_{5\text{-}6}$' denote average intramolecular ^{13}C discrimination of glucose C-1 to C-3, and C-5 to C-6, respectively. Glucose was extracted across an annually resolved tree-ring timeseries of *Pinus nigra* (1961-1995).

Dataset 2. Coefficients of correlation of isotope-climate relationships.

	SPEI$_1$ ~		*SPEI*$_3$ ~		*SPEI*$_6$ ~		*SPEI*$_8$ ~		*SPEI*$_{12}$ ~		*SPEI*$_{16}$ ~		*SPEI*$_{24}$ ~		*SPEI*$_{36}$ ~		*SPEI*$_{48}$ ~	
	Δ_{1-3}'	Δ_{5-6}'	Δ_{1-3}'	Δ_{5-6}'	Δ_{1-3}'	Δ_{5-6}'	Δ_{1-3}'	Δ_{5-6}'	Δ_{1-3}'	Δ_{5-6}'	Δ_{1-3}'	Δ_{5-6}'	Δ_{1-3}'	Δ_{5-6}'	Δ_{1-3}'	Δ_{5-6}'	Δ_{1-3}'	Δ_{5-6}'
MAMJ	0.54	0.19	0.49	0.18	0.35	0.12	0.38	0.08	0.32	-0.04	0.31	-0.11	0.30	0.06	0.35	0.12	0.35	0.20
MAMJJ	0.57	0.40	0.56	0.33	0.43	0.23	0.44	0.21	0.41	0.07	0.36	-0.04	0.35	0.10	0.40	0.15	0.37	0.24
MAMJJA	0.63	0.36	0.59	0.38	0.49	0.29	0.50	0.27	0.49	0.16	0.39	0.01	0.38	0.12	0.44	0.17	0.40	0.28
MAMJJAS	0.66	0.33	0.61	0.41	0.55	0.34	0.54	0.31	0.54	0.21	0.44	0.06	0.42	0.12	0.47	0.18	0.42	0.30
MAMJJASO	0.63	0.31	0.64	0.37	0.58	0.38	0.57	0.33	0.56	0.24	0.48	0.09	0.45	0.11	0.49	0.19	0.43	0.31
MAMJJASON	0.57	0.29	0.63	0.34	0.59	0.39	0.60	0.36	0.58	0.28	0.52	0.12	0.46	0.12	0.49	0.19	0.44	0.31
AMJJ	0.56	0.60	0.59	0.42	0.49	0.31	0.47	0.29	0.47	0.16	0.41	0.02	0.39	0.15	0.45	0.17	0.39	0.28
AMJJA	0.63	0.54	0.62	0.45	0.54	0.36	0.52	0.34	0.54	0.24	0.44	0.07	0.43	0.16	0.49	0.19	0.41	0.31
AMJJAS	0.66	0.50	0.64	0.48	0.58	0.40	0.56	0.36	0.58	0.28	0.49	0.12	0.46	0.15	0.51	0.19	0.43	0.33
AMJJASO	0.61	0.49	0.67	0.43	0.61	0.43	0.59	0.38	0.59	0.30	0.52	0.14	0.48	0.14	0.52	0.20	0.44	0.33
AMJJASON	0.56	0.47	0.66	0.40	0.62	0.44	0.60	0.40	0.60	0.33	0.55	0.17	0.49	0.14	0.53	0.21	0.45	0.33
MJJA	0.57	0.53	0.62	0.53	0.59	0.44	0.54	0.40	0.59	0.32	0.48	0.13	0.46	0.20	0.52	0.21	0.41	0.34
MJJAS	0.62	0.51	0.64	0.54	0.62	0.47	0.57	0.41	0.61	0.35	0.52	0.18	0.49	0.18	0.54	0.21	0.43	0.36
MJJASO	0.57	0.52	0.67	0.49	0.63	0.49	0.60	0.42	0.62	0.36	0.56	0.20	0.51	0.17	0.55	0.22	0.45	0.36
MJJASON	0.50	0.48	0.66	0.46	0.63	0.49	0.61	0.44	0.63	0.38	0.58	0.22	0.52	0.17	0.55	0.22	0.45	0.36
JJAS	0.52	0.33	0.60	0.55	0.63	0.47	0.58	0.43	0.61	0.37	0.54	0.22	0.51	0.18	0.57	0.22	0.45	0.38
JJASO	0.43	0.29	0.63	0.49	0.64	0.49	0.61	0.43	0.62	0.37	0.58	0.23	0.53	0.16	0.57	0.22	0.46	0.38
JJASON	0.35	0.26	0.62	0.45	0.63	0.48	0.62	0.45	0.62	0.39	0.60	0.26	0.53	0.16	0.57	0.23	0.47	0.37
JASO	0.33	0.26	0.56	0.44	0.64	0.51	0.62	0.46	0.61	0.40	0.60	0.28	0.54	0.16	0.58	0.24	0.47	0.40
JASON	0.25	0.23	0.53	0.38	0.62	0.49	0.62	0.48	0.61	0.42	0.62	0.30	0.54	0.16	0.57	0.24	0.48	0.38
ASON	0.10	-0.14	0.44	0.19	0.62	0.48	0.64	0.45	0.60	0.41	0.64	0.31	0.56	0.13	0.58	0.24	0.49	0.38

Climate parameters: *SPEI*$_i$, standardised precipitation-evapotranspiration index of different periods (i = 1, 3, 6, 8, 12, 16, 24, 36, 48 months). Climate data were averaged for all ≥ 4-month periods of the growing season (March to November). Months were abbreviated by their initial letters. Δ_{1-3}' and Δ_{5-6}' denote average intramolecular ^{13}C discrimination of glucose C-1 to C-3, and C-5 to C-6, respectively. Glucose was extracted across an annually resolved tree-ring timeseries of *Pinus nigra* (1961-1995).