

Reconciling intrinsic and ecosystem water-use efficiency reveals physiological mechanisms underlying carbon–water coupling across biological scales

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Abstract

Understanding how carbon–water coupling scales from leaves to ecosystems remains a major challenge because water-use efficiency (WUE) metrics integrate physiological, hydrological, and ecological processes. We integrated tree-ring width, triple stable isotopes ($\delta^{13}\text{C}$, $\delta^{18}\text{O}$ and $\delta^2\text{H}$), eddy covariance (EC) observations, and a process-based model to investigate the drivers of intrinsic ($i\text{WUE}$) and ecosystem water-use efficiency (WUE_{eco}) across biological scales in a mature *Fagus sylvatica* forest between 2004 and 2023. Modelled and isotope-derived $i\text{WUE}$ were strongly correlated ($r = 0.61$, $p < 0.05$) and both indicated an approximately 20% long-term increase, consistent with increasing gross primary productivity. In contrast, WUE_{eco} derived from EC was lower than model estimates and exhibited a positive trend not reproduced by the model. This divergence primarily reflected differences in biological representation and hydrological integration, as the single-species model underestimated ecosystem evapotranspiration by excluding co-occurring deciduous and evergreen species together with understory vegetation. Triple-isotope analyses showed that the long-term increase in $i\text{WUE}$ was driven by enhanced photosynthetic assimilation rather than sustained stomatal closure, whereas $\delta^{18}\text{O}$ and $\delta^2\text{H}$ variability was mainly controlled by source-water dynamics. These findings provide a mechanistic framework for reconciling leaf- and ecosystem-scale WUE and improving predictions of forest carbon–water coupling under climate change.

Introduction

Understanding how forests coordinate carbon uptake and water loss is central to predicting terrestrial ecosystem responses to climate change. Carbon assimilation through photosynthesis and water loss through transpiration are tightly coupled via stomatal regulation, making water-use efficiency (WUE) a key integrative metric of plant physiological functioning. WUE describes the balance between carbon gain and water loss and therefore reflects how vegetation optimizes resource use under changing environmental conditions (Farquhar et al., 1982; Medlyn et al., 2017). At regional and global scales, shifts in WUE can strongly influence carbon sequestration, evapotranspiration (ET), and land–atmosphere feedbacks, making this parameter critical for

67 constraining Earth System Models and forecasting ecosystem responses to rising atmospheric CO₂
68 and intensifying hydroclimatic stress (Keenan et al., 2013; Peters et al., 2018).

69 However, WUE is not a scale-invariant property, and its interpretation depends strongly on the
70 spatial and temporal scale at which it is measured (Beer et al., 2009). Indeed, although WUE is
71 widely adopted within the scientific community as a key indicator of plant performance under
72 water-limited conditions, its meaning has progressively broadened over time. As a result, WUE is
73 now characterized by multiple, coexisting definitions, each tailored to specific methodological
74 approaches or research contexts. While this conceptual flexibility has facilitated its application
75 across diverse disciplines, it has also led to a certain degree of ambiguity, ultimately limiting the
76 comparability of results across studies and contexts (Puchi et al., 2025). At the leaf level, intrinsic
77 water-use efficiency ($iWUE$) is defined as the ratio between net photosynthetic assimilation (A) and
78 stomatal conductance to water vapor (g_s). Tree-ring $\delta^{13}C$ provides a retrospective archive of $iWUE$
79 because carbon isotope discrimination during photosynthesis reflects the ratio of intercellular to
80 atmospheric CO₂ concentrations (c_i/c_a), which is controlled by the balance between assimilation and
81 stomatal conductance (Farquhar et al., 1982). Consequently, isotope-derived $iWUE$ integrates
82 physiological regulation over the period of wood formation and provides a powerful tool to
83 reconstruct long-term plant responses to environmental variability and atmospheric CO₂ increase
84 (Saurer & Voelker, 2022).

85 In contrast, ecosystem-scale WUE (WUE_{eco}) is typically quantified using eddy covariance
86 measurements as the ratio between gross primary productivity (GPP) and evapotranspiration. This
87 flux-based metric integrates canopy-level carbon uptake and total ecosystem water losses, including
88 transpiration, soil evaporation, and canopy interception (Knauer et al., 2018). As such, WUE_{eco}
89 represents an emergent property of vegetation structure, atmospheric demand, soil hydrology, and
90 land–atmosphere coupling. Critically, the inclusion of non-transpirational water fluxes in ET means
91 that WUE_{eco} does not only reflect stomatal-level physiological regulation, potentially leading to
92 systematic biases when comparing with leaf-level metrics (Dekker et al., 2016; Wang et al., 2020;
93 see Puchi et al. (2025) for a broad overview of the many definitions and equations used).

94 Bridging leaf-level and ecosystem-level representations of WUE, therefore, remains a major
95 challenge in ecohydrology and ecosystem modeling. Several fundamental issues complicate cross-
96 scale comparisons: (1) differences in temporal integration of tree rings record seasonal to annual
97 signals, while eddy covariance provides sub-daily to daily measurements; (2) spatial
98 representativeness individual trees vs. ecosystem-level fluxes; and (3) hydrological partitioning
99 stomatal transpiration vs. total evapotranspiration (Medlyn et al., 2017). Several studies have

reported weak or inconsistent relationships between long-term trends in $\delta^{13}\text{C}$ inferred from tree rings and WUE_{eco} derived from eddy covariance measurements (Seibt et al., 2008; Gea-Izquierdo et al., 2015; Lavergne et al., 2019; Li et al., 2022). These discrepancies highlight that processes measured at the stomatal scale are not directly comparable to those emerging at the ecosystem scale, where non-stomatal water fluxes and canopy structural effects become increasingly important.

Therefore, while partitioning ET into transpiration and evaporation components could theoretically improve cross-scale comparisons, such partitioning remains challenging and uncertain, particularly in complex forest ecosystems (Nelson et al., 2018). Therefore, understanding the relationship between leaf-level $\delta^{13}\text{C}$ and ecosystem-level WUE calculated from total ET remains a fundamental challenge for interpreting tree-ring isotope records in the context of ecosystem functioning.

Additionally, increases in $\delta^{13}\text{C}$ do not always correspond to higher forest growth or productivity, because the relationship between WUE and carbon allocation depends strongly on hydroclimatic constraints such as vapor pressure deficit (VPD), soil moisture availability, and plant hydraulic regulation (Frank et al., 2015; Zhang et al., 2025). Among these factors, VPD plays a central role in linking leaf- and ecosystem-scale WUE because it simultaneously influences stomatal conductance and atmospheric evaporative demand. As VPD increases, transpiration may continue to rise despite partial stomatal closure owing to the steeper leaf-to-air vapor pressure gradient, potentially decoupling leaf-level physiological responses from ecosystem-scale carbon–water exchange (Grossiord et al., 2020; Diao et al., 2024; Medlyn et al., 2017). Consequently, understanding whether long-term changes in $\delta^{13}\text{C}$ are driven primarily by changes in g_s or A is critical for interpreting isotope records in the context of ecosystem functioning (Guerrieri et al., 2019). Because $\delta^{13}\text{C}$ reflects changes in c_i/c_a rather than A or g_s independently, additional constraints are often required to distinguish whether observed $\delta^{13}\text{C}$ trends arise from shifts in photosynthetic capacity or stomatal regulation (Scheidegger et al., 2000). In particular, oxygen isotopes ($\delta^{18}\text{O}$), which are primarily sensitive to transpiration and evaporative enrichment of leaf water (Roden et al., 2000; Barbour, 2007), have been combined with $\delta^{13}\text{C}$ records within the conceptual dual-isotope framework proposed by Scheidegger et al. (2000) to attribute $\delta^{13}\text{C}$ changes to either stomatal or biochemical drivers. More recently, hydrogen isotopes ($\delta^2\text{H}$) have also been explored as complementary tracers of leaf water enrichment and source-water signals, providing additional constraints on plant water relations and ecohydrological processes that can further refine interpretations of stomatal regulation and transpiration dynamics (Lehmann et al., 2021; Saurer et al., 2025). This approach has since been applied in dendroecological studies to interpret long-term

133 isotope trends under changing atmospheric and climatic conditions (e.g., Saurer et al., 2004;
134 Lavergne et al., 2020).

135 Process-based ecosystem models, such as 3D-CMCC-FEM, provide a promising framework for
136 bridging leaf-level and ecosystem-level WUE estimates because they explicitly simulate canopy
137 photosynthesis, stomatal conductance, transpiration, carbon allocation, respiration, and water
138 balance using mechanistic eco-physiological principles (Fatichi et al., 2016; Keenan et al., 2013;
139 Collalti et al., 2016; 2024; Saponaro et al., 2026). By systematically comparing model simulations
140 with both tree-ring isotope records and eddy covariance observations, we can assess the extent to
141 which models realistically represent the physiological processes driving WUE variability across
142 spatial and temporal scales, while also using models to bridge observational gaps by inferring
143 processes that are difficult or impossible to measure directly. This integrative approach also enables
144 the identification of key mechanisms underlying discrepancies between leaf-level and ecosystem-
145 level estimates, thereby helping to clarify scale-dependent dynamics and improve model
146 performance.

147 Recent studies have increasingly adopted multi-scale approaches to investigate the responses of
148 WUE to environmental stress. For example, Rambal et al. (2025) combined stable isotopes, gas
149 exchange, and eddy covariance measurements to assess drought effects on intrinsic and ecosystem
150 water-use efficiency in a Mediterranean evergreen oak forest, highlighting the importance of
151 considering biological scale when interpreting carbon–water relationships. However, despite these
152 advances, studies integrating physiological proxies, ecosystem observations, and mechanistic
153 modelling within a unified framework remain lacking.

154 In this study, we integrate eddy covariance observations, tree-ring stable isotopes ($\delta^{13}\text{C}$, $\delta^{18}\text{O}$, $\delta^2\text{H}$),
155 dendrochronological measurements, and simulations from a process-based model to investigate
156 carbon–water interactions in a temperate mixed forest dominated by *Fagus sylvatica* L. at the
157 Lägeren site in Switzerland over a period of two decades (2004–2023). Specifically, this study
158 addresses three main objectives:

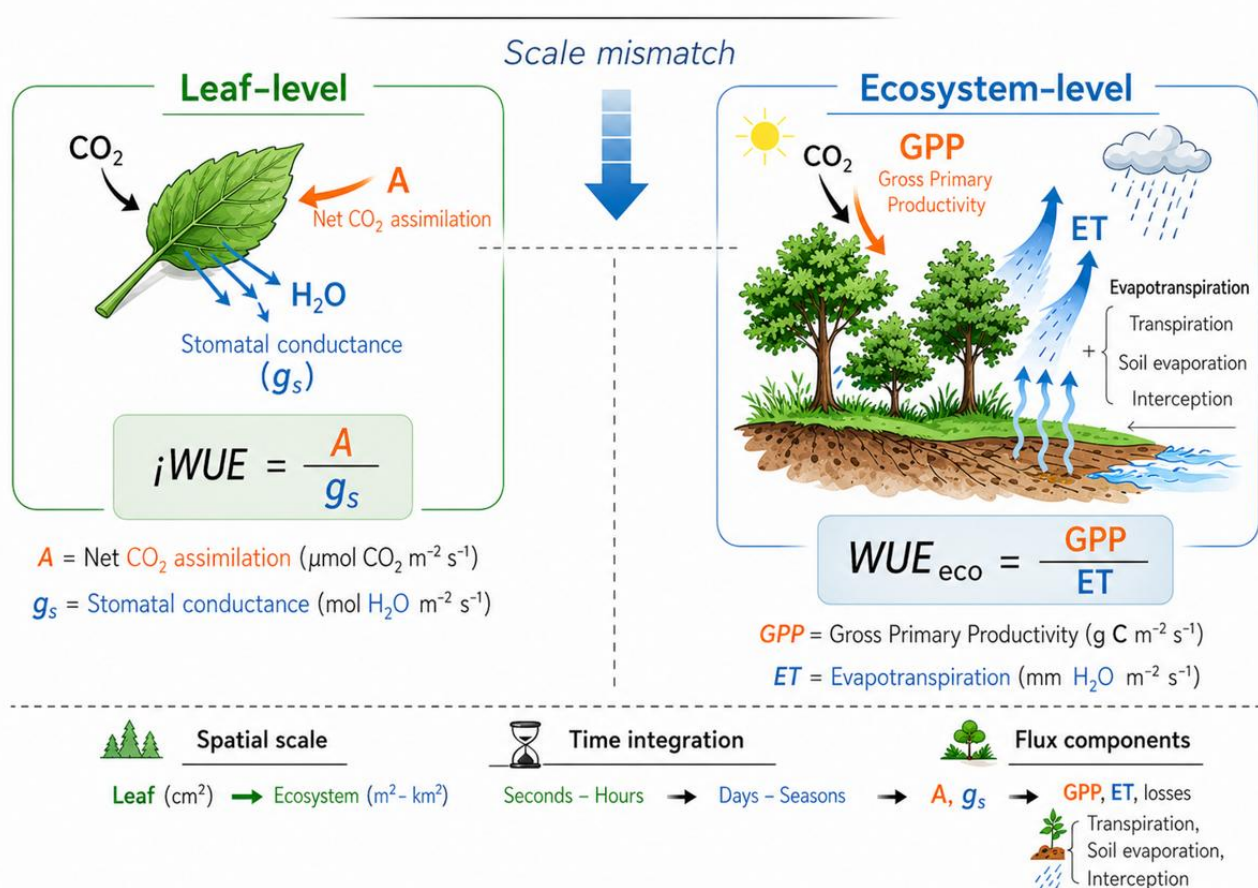
159 1) *Temporal scaling and integration*: To evaluate whether the agreement between isotope-derived
160 intrinsic $i\text{WUE}$ (i.e. A/g_s) and ecosystem WUE_{eco} (i.e. GPP/ET) improves with temporal aggregation
161 (daily $\rightarrow$ monthly $\rightarrow$ annual), and to assess whether restricting analyses to the growing season
162 enhances cross-scale comparability (see Box 1);

163 2) *Model-data integration for bridging scales*: To use a process-based model as a mechanistic
164 bridge between leaf-level and ecosystem-level WUE by: (i) validating model-simulated GPP and

ET against eddy covariance observations, (ii) comparing model-derived $iWUE^{\text{mod}}$ with tree-ring isotope-derived $iWUE^{\text{obs}}$, and (iii) evaluating whether the model can reproduce observed patterns in both leaf-level and ecosystem-level WUE;

3) *Disentangling physiological controls*: To assess the relative contributions of stomatal conductance and photosynthetic assimilation in driving interannual variability in intrinsic $iWUE$, using simulations from the process-based model together with the conceptual dual-isotope framework (Scheidegger et al., 2000). Specifically, the model was used to simulate physiological variables (e.g., stomatal conductance), while the combined interpretation of tree-ring $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ was used to infer whether changes in $iWUE$ were primarily driven by stomatal regulation or by changes in photosynthetic capacity.

By integrating complementary methodologies operating at different levels of ecological organization, from tree-ring stable isotopes and dendrochronology at the leaf level to eddy covariance observations and process-based ecosystem modelling at the ecosystem level, this study presents, to our knowledge, the first mechanistic framework for disentangling WUE across biological scales. Bridging physiological and ecosystem processes may prove essential for advancing our understanding of carbon–water coupling in a changing climate.



Box 1. Conceptual framework illustrating the scale mismatch between intrinsic water-use efficiency ($iWUE$) at the leaf level and ecosystem water-use efficiency (WUE_{eco}). At the leaf scale, $iWUE$ is defined as the ratio between net CO_2 assimilation (A) and stomatal conductance to water vapor (g_s), reflecting the physiological trade-off between carbon gain and water loss regulated by stomata. At the ecosystem scale, WUE_{eco} is defined as the ratio of gross primary productivity (GPP) to evapotranspiration (ET), integrating canopy-level carbon uptake and total water fluxes, including transpiration, soil evaporation, and interception losses. The schematic highlights the conceptual and process-based differences between scales, emphasizing that $iWUE$ and WUE_{eco} are governed by distinct flux components, integration times, and spatial domains.

Materials and methods

Study site and climate

The study was conducted in a managed deciduous mountain forest located on the steep slopes of the Lägeren mountain (northwest of Zurich, Swiss Plateau), at approximately 700 m asl. The forest within the tower footprint is characterized by a mixed-species deciduous stand. The most abundant tree species are the European beech (*Fagus sylvatica* L.), ash (*Fraxinus excelsior* L.), and Norway spruce (*Picea abies* (L.) H.Karst., 1881) (Shekhar et al., 2024). In spring and early summer, bear's garlic (*Allium ursinum* (L., 1753)) forms a dense understory (Etzold et al., 2011). The site, known as CH-Lae in the global FLUXNET network, has been continuously monitored for carbon, water, and energy fluxes using the eddy covariance technique since April 2004. The tower is located at 47°28'42.0" N, 8°21'51.8" E, at an elevation of 689 m a.s.l. (Fig. 1a).

The site is underlain by limestone bedrock, and the main soil types are Rendzic Leptosols (Rendzinas) and Haplic Cambisols, according to the World Reference Base for Soil Resources (IUSS Working Group WRB, 2007). Soils are well-drained, and the litter layer is generally thin, as leaf litter decomposes almost completely within a year due to the relatively mild climatic conditions and high biological activity.

The CH-Lae site is characterized by a temperate humid climate with mild temperatures and abundant precipitation throughout the year. Based on data recorded from 2004 to 2023, the mean annual air temperature is 8.7 °C, and the mean annual precipitation totals approximately 870 mm. Precipitation is relatively evenly distributed across months, with slightly wetter conditions during late spring and summer. The warmest month is July, with a mean temperature of about 22 °C, while

the coldest month is January, averaging around -2°C . Snowfall occurs occasionally in winter but is generally short-lived due to moderate temperatures (Fig. 1b).

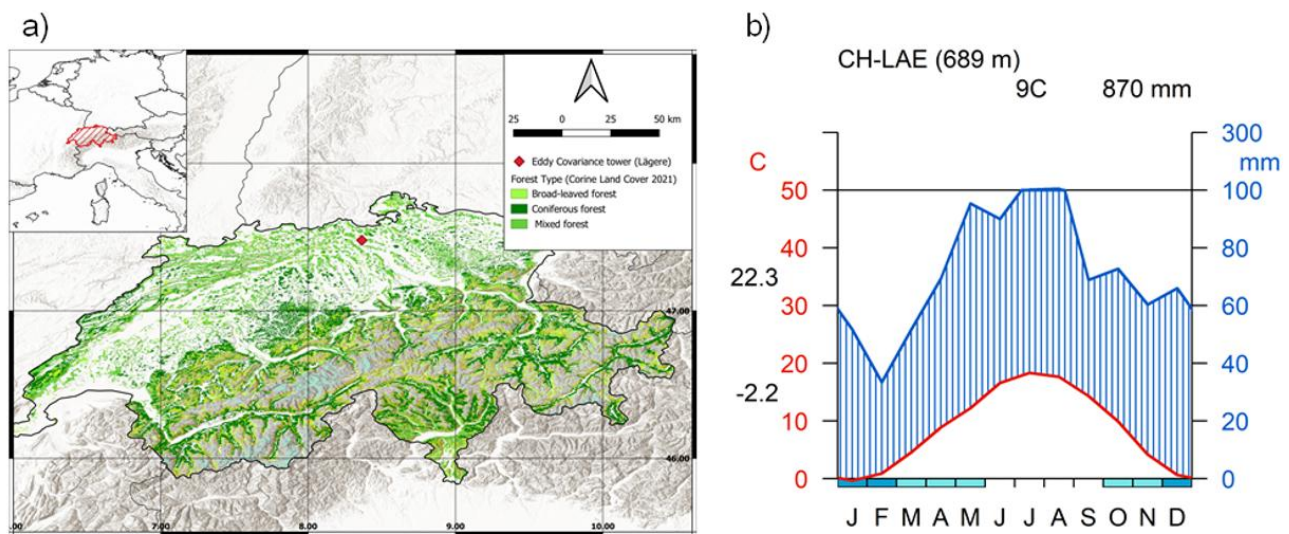


Fig. 1 (a) Location of Lägeren CH-Lae site, Canton of Aargau, Switzerland, showing forest cover types from CORINE Land Cover (2021). The red dot indicates the position of the eddy covariance (EC) tower; (b) Walter-Lieth climate diagram for the period 2004-2023, derived from meteorological data recorded at the EC tower. Teal boxes (bottom) are frost indicators: lighter boxes mark months with *probable frost* (mean minimum temperature approaches 0°C); darker boxes mark months with *certain frost* (mean minimum temperature below 0°C). In this diagram, frost risk is concentrated in J, F, M, A, N, D, consistent with the recorded minimum of -2.2°C .

223

2.2 Eddy covariance flux measurements

Half-hourly fluxes of energy, water vapour and carbon dioxide (CO_2) have been continuously measured using the eddy covariance (EC) method since April 2004 (Etzold et al., 2010). The EC system consisted of a three-dimensional sonic anemometer (Gill Solent HS, Gill Instruments Ltd., Lymington, UK) for the measurements of high-frequency (20Hz) wind data, and an open-path (model LI-7500, LI-COR Biosciences, Lincoln, NE, USA; installed between 2004 and 2015) or enclosed-path (LI-7200, from 2016 onwards) infrared gas analyzer (IRGA). The EC instruments were mounted at a height of 47 m above ground, corresponding to approximately 17 m above the mean canopy height. Fluxes were calculated following established community guidelines (Aubinet et al., 2012; Sabbatini et al., 2018). Subsequent data quality control, gap-filling, and flux partitioning into gross primary production (GPP) and ecosystem respiration (R_{eco}) followed the

standard FLUXNET approach (Pastorello et al., 2020). Detailed site information is available from the Swiss FluxNet network (<https://www.swissfluxnet.ethz.ch>, site CH-Lae).

Evapotranspiration (ET) was derived from latent heat flux (LE) measurements obtained from the EC system. Half-hourly LE values (W m^{-2}) were converted to ET using the latent heat of vaporization of water ($\lambda = 2.45 \times 10^6 \text{ J kg}^{-1}$) according to:

$$ET = LE / \lambda \quad \text{Eqn 1}$$

where ET is expressed in $\text{kg m}^{-2} \text{ s}^{-1}$, equivalent to mm s^{-1} . Half-hourly ET estimates were integrated and aggregated to obtain daily ET totals (mm day^{-1}).

The upper platform of the tower is equipped with instruments that record standard meteorological variables, including net radiation (Kipp & Zonen CNR1, Delft, Netherlands), air temperature, and relative humidity (Rotronic MP101A, Bassersdorf, Switzerland). In the vicinity of the flux tower, soil temperature and moisture are monitored at a depth of 5 cm using ECH₂O EC-20 probes (Decagon Devices, Pullman, WA, USA). Soil heat flux is measured with two HFP01 heat flux plates (Hukseflux, Delft, Netherlands) installed side by side at 3 cm depth. Mean soil heat flux is corrected for heat storage changes in the layer above the plates following the procedure described by Monteith and Unsworth (1990). All measurements are automatically controlled and recorded by a CR10X data logger (Campbell Scientific Inc., Loughborough, UK).

2.3 Field sampling and processing dendrochronological data

In July 2024, 15 trees of *Fagus sylvatica* were sampled within the eddy covariance tower footprint, targeting the upslope sector of the stand where beech dominance is highest (approximately 85% of basal area). Cores were extracted at 1.3 m from the ground using a 5 mm increment borer, with two cores extracted per tree. In the tree-ring laboratory of the Federal Swiss Institute for Forest, Snow, and Landscape Research (WSL), wood cores were air-dried and polished with sandpaper of successively increasing grains to visualize the ring boundaries. Ring widths were measured to a precision of 0.01 mm using the TSAP measuring device (Rinntech). Tree-ring width (TRW) series were then visually cross-dated using standard dendrochronological methods (Stokes and Smiley, 1968) and checked for dating accuracy and measurement errors with the COFECHA program (Holmes, 1983). The tree-ring chronology was performed using the R-package ‘dplR’ (Bunn, 2008; R Core Team, 2022).

268 2.4 Triple isotope data

269 For stable isotope analysis, cores from five trees were selected, avoiding cores with visible defects.
 270 Measurements of $\delta^{13}\text{C}$, $\delta^{18}\text{O}$, $\delta^2\text{H}$ were conducted separately for each selected tree and each year
 271 over the period 2000-2023. We split off the individual years with a razor blade under a
 272 stereomicroscope. For each year and tree, we pooled wood material from both cores per tree for a
 273 more representative tree value and to ensure sufficient material for measurement of small rings. The
 274 separated tree rings were transferred into filter bags (Ankom Technology, Macedon, NY, USA) for
 275 subsequent cellulose extraction. We performed cellulose extraction according to Boettger et al.
 276 (2007) and cellulose homogenization following Laumer et al. (2009). Cellulose samples were
 277 weighed (0.9-1.1 mg) into silver capsules, equilibrated with water vapor (Schuler et al., 2022), and
 278 then converted to H_2 and CO by pyrolysis at 1420°C (PYRO-cube, Elementar, Hanau, Germany) for
 279 subsequent measurements of isotope ratios with an isotope-ratio mass spectrometer (IRMS; Delta
 280 Plus XP; Thermo Finnigan Mat, Bremen, Germany) with a precision $\pm 0.2\text{‰}$ for $\delta^{13}\text{C}$, $\pm 0.3\text{‰}$ for
 281 $\delta^{18}\text{O}$ and $\delta^2\text{H}$ 3.0‰ (Saurer et al., 2025).

282 In this study, $\delta^{13}\text{C}$ was used as a proxy for intrinsic water-use efficiency and photosynthetic carbon
 283 assimilation (Farquhar et al., 1982; Scheidegger et al., 2000), $\delta^{18}\text{O}$ as a proxy integrating source-
 284 water isotopic composition and evaporative leaf water enrichment (Roden et al., 2000; Barbour,
 285 2007), and $\delta^2\text{H}$ primarily as a proxy for source-water isotopic variability and physiological
 286 responses (Lehmann et al., 2021; Saurer et al., 2025).

287

288 2.5 $i\text{WUE}$ inferred from carbon isotope discrimination

289 Measurements of $\delta^{13}\text{C}$ were used to calculate $i\text{WUE}$ for European beech trees. $i\text{WUE}$ is defined as
 290 the ratio between the net photosynthesis rate (A) and the stomatal conductance to water vapor (g_s)
 291 rate (Ehleringer & Dawson, 1992) and was computed as per Eqn 2:

292

$$293 \quad i\text{WUE} = A/g_s = (c_a - c_i) / 1.6, \quad \text{Eqn 2}$$

294

295 where c_a is the atmospheric CO_2 concentration, c_i is the intercellular CO_2 concentration (both in
 296 ppmv), and 1.6 represents the ratio of the diffusivities of water vapour and CO_2 in air.

For European beech, $iWUE$ can be derived from the carbon isotope composition ($\delta^{13}C_{tree}$) of plant material, which is related to the isotopic composition of atmospheric CO_2 ($\delta^{13}C_{atm}$) and to the c_i/c_a ratio, following the model by Farquhar and Richards (1984) and Farquhar et al. (1982) (Eqn 3):

$$\delta^{13}C_{tree} = \delta^{13}C_{atm} - a - [(b - a) c_i] / c_a, \quad \text{Eqn 3}$$

where a is the fractionation factor during diffusion through stomata (4.4‰) and b is the fractionation factor associated with Rubisco carboxylation (27‰). From Eqn 2, c_i can be derived as (Eqn 4):

$$c_i = c_a (\delta^{13}C_{atm} - \delta^{13}C_{tree} - a) / (b - a). \quad \text{Eqn 4}$$

Finally, the $iWUE$ is obtained as (Eqn 5):

$$iWUE = c_a (b - \delta^{13}C_{atm} + \delta^{13}C_{tree}) / 1.6 (b - a). \quad \text{Eqn 5}$$

Values of $\delta^{13}C_{atm}$ and the atmospheric concentration of CO_2 for the period 2000–2023 were obtained from the Mauna Loa station data (available at <http://www.esrl.noaa.gov/>).

2.6 Modelling C and water cycle

Carbon and water fluxes were simulated using the 3D-CMCC-FEM (“*Three Dimensional - Coupled Model Carbon Cycle - Forest Ecosystem Module*”) (Collalti et al., 2016; 2020; 2024; Dalmonech et al., 2022; 2024; Puchi et al., 2026; Saponaro et al., 2025; 2026). The 3D-CMCC-FEM is a biogeochemical, biophysical and eco-physiological process-based model designed to simulate carbon and water cycle dynamics in forest ecosystems. The model has been extensively validated across species and climatic gradients within e.g., the Fluxnet, ICOS, and PROFOUND/ISIMIP networks (e.g., Marconi et al., 2017; Reyer et al., 2019, 2020; Mahnken et al., 2022).

Model simulations were used to estimate gross primary productivity (GPP), evapotranspiration (ET), intrinsic water-use efficiency ($iWUE$; the ratio of net carbon assimilation, A , to stomatal conductance for water vapour, g_s), and ecosystem water-use efficiency (WUE_{eco} ; the ratio of GPP to ET). Simulated fluxes were compared with eddy-covariance observations and tree-ring isotope-derived estimates to evaluate the consistency of carbon–water coupling across daily, monthly and annual timescales.

Photosynthesis (A , $\mu\text{mol } CO_2 \text{ m}^{-2} \text{ s}^{-1}$) is computed according to the biochemical model of Farquhar et al. (1980) with temperature acclimation as in Kattge & Knorr (2007) and soil moisture

326 regulation as in Bonan et al. (2011). A is calculated as the minimum of two potential limitations
327 (Eqn 6):

$$328$$

$$329 \quad A = \min(A_v, A_j) \quad \text{Eqn 6}$$

330
331 where A_v is Rubisco-limited assimilation, and A_j is electron transport-limited assimilation, ,
332 computed respectively as (Eqn 7 and Eqn 8):

$$333$$

$$334 \quad A_v = (V_{cmax} \cdot (c_i - \Gamma) / (c_i + K_m)) - R_d^* \quad \text{Eqn 7}$$

335
336 where V_{cmax} is the maximum carboxylation rate corrected for temperature and soil water
337 availability, c_i is intercellular CO_2 concentration, Γ is the CO_2 compensation point, K_m is the
338 Michaelis-Menten coefficient, and R_d is mitochondrial respiration in the light.

$$339$$

$$340 \quad A_j = (J \cdot (c_i - \Gamma) / (4.5 \cdot c_i + 10.5 \cdot \Gamma)) - R_d^{**} \quad \text{Eqn 8}$$

341
342 where J is the electron transport rate, and the empirical coefficients (4.5 and 10.5, respectively)
343 follow Bernacchi et al. (2003).

344 Both V_{cmax} and J_{max} (defined at 25°C) are dynamically adjusted using temperature response
345 functions (Medlyn et al., 2002) and further downregulated by soil water content (Bonan et al.,
346 2011). These parameters are computed separately for sunlit and shaded canopy fractions,
347 incorporating nitrogen variation across canopy layers to ensure physiologically realistic limitations
348 to photosynthesis through time.

349 Stomatal conductance (g_s) is calculated daily following the multiplicative stomatal regulation model
350 of Jarvis (1976) (Eqn 9):

$$351$$

$$352 \quad g_s = g_{smax} \cdot f_Q \cdot f_\psi \cdot f_T \cdot f_{VPD} \cdot f_{\text{CO}_2} \cdot f_{Age} \cdot f_{CT} \quad \text{Eqn 9}$$

353

where $g_{s,max}$ is the maximum potential stomatal conductance and f terms represent environmental and physiological modifiers (light, soil water potential, air temperature, vapor pressure deficit, atmospheric CO₂ concentration, age, and temperature-pressure correction) varying from 0 (totally limitant) to 1 (not limitant), with the exception for f_{CO_2} , as described in Collalti et al. (2018; 2024) and Saponaro et al. (2026).

Intrinsic water-use efficiency is then derived from the ratio of A (Eqn 6) to g_s (Eqn 9) as (Eqn 10):

$$iWUE = \frac{A}{g_s} \quad \text{Eqn 10}$$

Daily model-derived $iWUE^{mod}$ was averaged over the vegetative period and subsequently aggregated to annual values to ensure consistency with the annual integration of isotope-derived $iWUE^{obs}$.

In the 3D-CMCC-FEM, gross primary productivity (GPP) is obtained by integrating canopy photosynthesis over time, while total evapotranspiration results from the combined simulation of canopy transpiration, soil evaporation, and interception/evaporation losses within the coupled water balance module. All evapotranspirative processes such as: canopy interception and evaporation (which includes also snow interception, melting and subsequent evaporation), stomatal/leaf/canopy transpiration, and soil evaporation, are computed at daily time steps using the Penman–Monteith equation as modified by Waring & Running (2007) and Monteith & Unsworth (2008). The formulation estimates latent heat fluxes as a function of incoming radiation, vapour pressure deficit, and surface conductances, based on meteorological conditions and surface characteristics (Collalti et al., 2024).

Soil moisture limitations directly constrain both photosynthetic assimilation and stomatal opening, thereby influencing $iWUE$ dynamics, while climatic drivers including air temperature, vapour pressure deficit (VPD), and atmospheric CO₂ concentration further modulate the trade-off between carbon gain and water loss through stomatal regulation (Puchi et al., 2026; Saponaro et al., 2026).

Model setup (Table 1), parameterization, and site-specific inputs (e.g., DBH-sapwood area relationships) are detailed in the Supplementary Material (Table S1). Simulations were performed using the R-package ‘R3DFEM’ (Collalti et al., 2022; Vangi et al., 2025).

Table 1 Biometric parameters of sampled *Fagus sylvatica* trees (N° of trees, height, DBH, TRW and age) and stand characteristics in 2004 for model initialization. Data are means $\pm$ standard deviation.

Variable	CH-Lae
N° of trees	15
Height (m)	25.2 $\pm$ 1.4
DBH (cm)	48.2 $\pm$ 1.4
TRW (mm)	0.97 $\pm$ 0.44
Age (year)	184 $\pm$ 18.7
Stand density (trees ha ⁻¹) 2004	213
Stand density (trees ha ⁻¹) 2012	237.6
Basal area (m ² ha ⁻¹)	48.15
Leaf area index (m ² m ⁻²)	4.1 $\pm$ 0.3
Soil drainage	well-drained

To validate the model, we first assessed the temporal relationships between modelled GPP and EC-derived GPP estimates across daily, seasonal, and interannual timescales (see Supplementary Notes S1; Figs. S1, S2, S3). This step confirmed that the simulated GPP dynamics reliably captured observed patterns, providing a robust basis for subsequent i WUE and WUE_{eco} analyses.

2.7. Statistical analyses and considerations

2.7.1 Data cleaning, filtering, and computation of WUE_{eco} (ratio-of-sums)

Daily eddy-covariance and model outputs were quality-controlled prior to analysis. To refine the temporal scale of the analysis between i WUE derived from isotopic measurements and simulated i WUE, we applied a daily response function using the DendroTools R package (Jevšenak and Levanič, 2018). During the analysis, the observed and simulated i WUE time series were linearly detrended using the Standard Linear Detrending (SLD) method implemented in the *daily_response()* function to remove long-term trends and emphasize interannual variability. Pearson correlation coefficients were then calculated over moving windows ranging from 30 to 150 days within the growing season (DOY 105–243) to identify the period of strongest agreement

between the two datasets. The highest positive correlation was observed between day of year (DOY) 172 (21 June) and DOY 214 (2 August) (Fig. S4). This interval largely overlaps with the June–August period previously identified as exhibiting the strongest climate–stable isotope relationships in *Fagus sylvatica* tree rings (Vitali et al., 2021). The identified period was subsequently used for comparisons between isotope-derived and modelled δ WUE (Fig. S4).

To ensure internal consistency of model fluxes, days with $GPP_{\text{model}} = 0$ were forced to $ET_{\text{model}} = 0$. Extreme physiological values were removed using a daily percentile filter: for each date, the 99th percentile (P99) of δ WUE and g_s was computed across all available records, and observations exceeding either threshold were discarded. Ecosystem water-use efficiency modelled ($WUE_{\text{eco}}^{\text{mod}}$) and obtained by eddy covariance observations ($WUE_{\text{eco}}^{\text{obs}}$) were derived from paired flux values with positive denominators, and daily values outside the range $0 < WUE_{\text{eco}} < 15 \text{ g C mm}^{-1} \text{ H}_2\text{O}$ were excluded to prevent spurious spikes associated with very low ET.

Ecosystem water-use efficiency was computed as (Eqn 11):

$$WUE_{\text{eco}} = \frac{GPP}{ET} \quad \text{Eqn 11}$$

using a ratio-of-sums approach:

$$WUE_{\text{eco}}^{\text{obs}} = \Sigma GPP^{\text{obs}} / \Sigma ET^{\text{obs}} \text{ and } WUE_{\text{eco}}^{\text{mod}} = \Sigma GPP^{\text{mod}} / \Sigma ET^{\text{mod}} \quad \text{Eqn12}$$

In this context ‘obs’ refers to eddy covariance measurements and ‘mod’ to model outputs, summing only over days with valid paired observations at daily, monthly, and annual scales. This approach avoids biases associated with averaging ratios of daily values (Ito & Inatomi, 2012) and enables cross-scale comparison between tree-level and ecosystem-level metrics. WUE_{eco} analyses were restricted to the growing season (15 April–31 August) for *Fagus sylvatica* at this site (Etzold et al. 2021). All statistical analyses were performed using R version 4.3.3 (R Core Team, 2024).

2.7.2 Leaf-level water-use efficiency: isotope-derived vs. modelled δ WUE

To evaluate the consistency between independent estimates of intrinsic water-use efficiency, isotope-derived $iWUE^{obs}$ was compared with model-simulated $iWUE^{mod}$ using Pearson correlation analysis and linear regression. Slope, coefficient of determination (R^2), and 95% confidence intervals were computed for each regression model, and statistical significance was assessed using two-tailed t -tests ($\alpha = 0.05$). Long-term temporal trends in both $iWUE$ estimates were quantified by fitting linear regression models with *year* as the explanatory variable, with the regression slope representing the annual rate of change. The fitted trends were compared to assess the consistency between observed and modelled long-term trajectories over the study period (2004–2023).

2.7.3 Ecosystem water-use efficiency: observed vs. modelled WUE_{eco}

Agreement between eddy covariance-derived WUE_{eco}^{obs} and model-simulated WUE_{eco}^{mod} was evaluated using Pearson correlation analysis at multiple temporal scales (daily, monthly, and annual) to assess whether carbon–water coupling improved with increasing temporal aggregation. Linear regression models were applied to quantify the correspondence between observed and simulated ecosystem fluxes, with slope, R^2 , and 95% confidence intervals reported for each scale. Days with missing paired observations were excluded prior to aggregation, following the ratio-of-sums approach described in Section 2.7.1.

2.7.4 Isotopic controls on $iWUE$ and radial growth

To interpret the physiological drivers of $iWUE$ interannual variability, Pearson correlation and linear regression analyses were performed between $iWUE^{obs}$ and the stable isotope ratios $\delta^{18}O$ and δ^2H measured in *F. sylvatica* tree-ring cellulose. These analyses were designed to distinguish between stomatal regulation and source water isotopic composition as the dominant controls on $iWUE$ variability, following the conceptual framework of Scheidegger et al. (2000). According to this model, the covariation between $\delta^{13}C$ and $\delta^{18}O$ can be used to disentangle the relative contributions of stomatal conductance (g_s) and mesophyll carboxylation capacity (A) to $iWUE$ variability: when both $\delta^{13}C$ and $\delta^{18}O$ increase simultaneously, stomatal closure is the dominant driver, whereas when $\delta^{13}C$ increases while $\delta^{18}O$ remains unchanged or decreases, enhanced photosynthetic capacity (A) is the primary mechanism. To further characterise isotope coupling, the relationship between $\delta^{13}C$ and $\delta^{18}O$ was assessed to evaluate carbon–oxygen isotope co-variation, and the $\delta^{18}O$ – δ^2H relationship was examined to test for consistency with the meteoric water line and

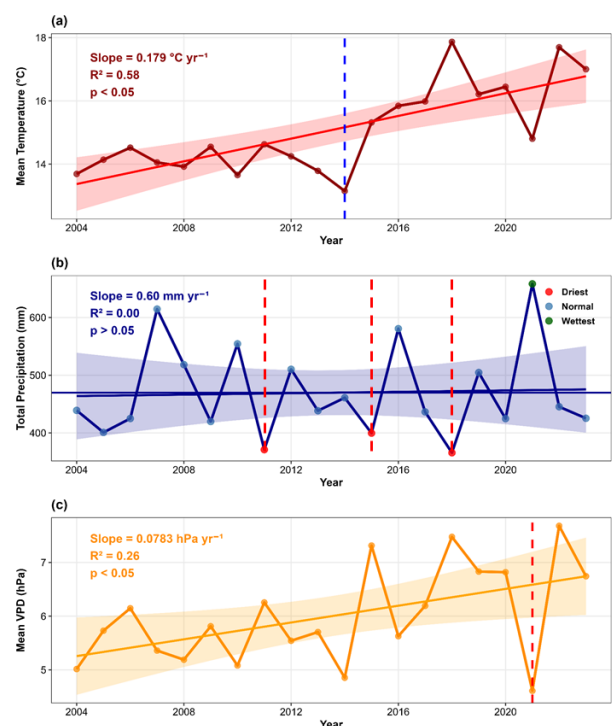
to evaluate the relative contribution of evaporative enrichment vs. source water dynamics to the oxygen and hydrogen isotope signals. Time series correlation between $\delta^{18}\text{O}$ and simulated ecosystem stomatal conductance (g_s) was additionally explored to identify physiological responses during climatically extreme years.

Finally, the relationship between TRW and isotope-derived $\delta^{18}\text{O}$ WUE was assessed using Pearson correlation and linear regression to evaluate whether long-term increases in water-use efficiency translated into enhanced radial growth. Additionally, the correlation between TRW and $\delta^2\text{H}$ was examined across the full time series (2000-2023) to identify years in which source water availability or metabolic fractionation processes dominated the growth response, with particular attention to the extreme climatic conditions.

3. Results

3.1 Climate trends during the growing season

Mean growing season temperature increased significantly at $0.179\text{ }^\circ\text{C yr}^{-1}$ ($R^2 = 0.58$, $p < 0.05$; Fig. 2a), totaling $\sim 3.4\text{ }^\circ\text{C}$ warming over the study period. The coldest growing season was 2014 ($13.0\text{ }^\circ\text{C}$, blue dashed line), while the warmest years were 2018 ($17.9\text{ }^\circ\text{C}$) and 2022 ($17.6\text{ }^\circ\text{C}$). Instead, precipitation showed no significant trend (slope = 0.60 mm yr^{-1} , $R^2 = 0.00$, $p > 0.05$; Fig. 2b), fluctuating around a mean of 470 mm . The driest years were 2018 (363 mm), 2011 (369 mm), and 2015 (401 mm , red circles), while the wettest year was 2021 (665 mm , green circle), representing a 41% increase above the mean. Interestingly, mean vapor pressure deficit (VPD) increased significantly at $0.0783\text{ hPa yr}^{-1}$ ($R^2 = 0.26$, $p < 0.05$; Fig. 2c), rising from $\sim 5.0\text{ hPa}$ (2004) to $\sim 6.8\text{ hPa}$ (2023), a 36% increase in atmospheric water demand. The lowest VPD occurred in 2021 (4.6 hPa , red dashed line), coinciding with the wettest growing season, while the highest VPD values were recorded in 2015 (7.2 hPa), 2018 (7.4 hPa), and 2022 (7.5 hPa). The combination of significant warming and increasing VPD without a corresponding precipitation trend indicates intensifying atmospheric water demand at the Lägeren site over the past two decades.



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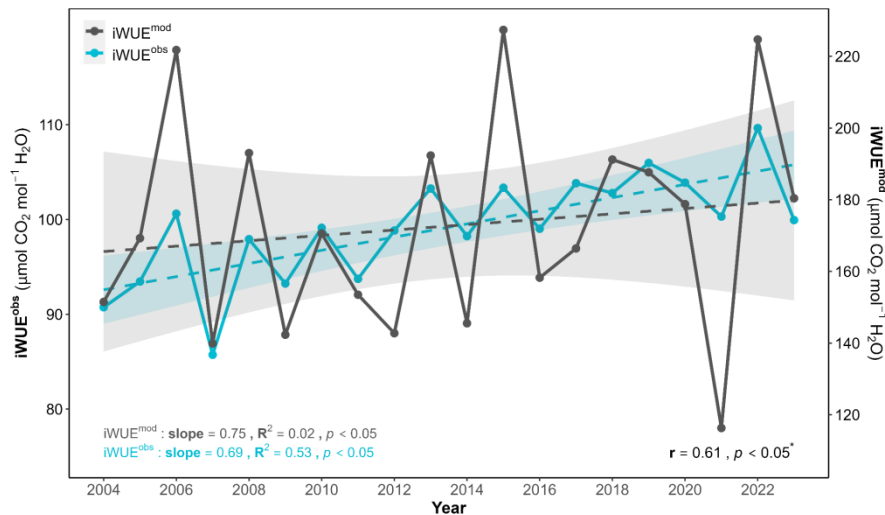
498 **Fig. 2** Climate trends during the growing season at the Lägeren CH-Lae flux tower data,
499 Switzerland, for the period 2004-2023. (a) Mean annual temperature with linear regression trend
500 line (red solid line) and 95% confidence interval (shaded area). The blue dashed vertical line
501 indicates the coldest year (2014). (b) Total precipitation with linear regression trend line (dark blue
502 solid line), mean precipitation reference line (horizontal dark blue line), and 95% confidence
503 interval (shaded area). Data points are color-coded according to precipitation extremes: red circles
504 indicate the three driest years, green circle indicates the wettest year (2021), and blue circles
505 represent normal years. (c) Mean annual VPD with linear regression trend line (orange solid line)
506 and 95% confidence interval (shaded area). The red dashed vertical line indicates the year with the
507 lowest VPD (2021). Regression parameters (slope, R², *p*-value) are displayed at the top left for each
508 variable.

509

510 3.2 Intrinsic water-use efficiency: model-isotope comparison

511 Annual intrinsic water-use efficiency derived from tree-ring isotopes (*i*WUE^{obs}) and simulated by
512 the 3D-CMCC-FEM model (*i*WUE^{mod}) were significantly correlated over the period 2004–2023 (*r* =
513 0.61, *p* < 0.05, *n* = 20; Fig. 3). The comparison was based on the optimal intra-annual window
514 identified using DendroTools (DOY 172–214; 21 June–2 August; see Methods), indicating that
515 model captures interannual variability in leaf-scale carbon-water coupling dynamics mediated by
516 stomatal regulation (Fig. 3). *i*WUE^{obs} averaged 98.9 ± 6.2 μmol CO₂ mol⁻¹ H₂ O, whereas

517 $iWUE^{mod}$ showed higher absolute values and substantially greater inter-annual variability (averaged
518 $172.7 \pm 30.3 \mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$). Both datasets exhibited significant positive trends over the
519 study period, with $iWUE^{mod}$ showing steeper increase (slope = $0.75 \mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$
520 year^{-1} , $R^2 = 0.02$, $p < 0.05$) compared to $iWUE^{obs}$ values (slope = $0.69 \mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$
521 year^{-1} , $R^2 = 0.53$, $p < 0.05$).



522

523 **Fig. 3** Inter-annual variability between $iWUE^{obs}$ (cyan line) and $iWUE^{mod}$ (gray line) from 21 June
524 to 2 August (DOY 172-214) for the period 2004-2023. Dashed lines represent linear regression
525 trends with shaded areas indicating 95% confidence intervals. Trend statistics (slope in $\mu\text{mol CO}_2$
526 $\text{mol}^{-1} \text{ H}_2\text{O year}^{-1}$, coefficient of determination R^2 , and p -value) are displayed adjacent to their
527 corresponding trend lines in matching colors. Overall Pearson correlation coefficient and
528 significance level between annual values are shown (bottom right, bold).

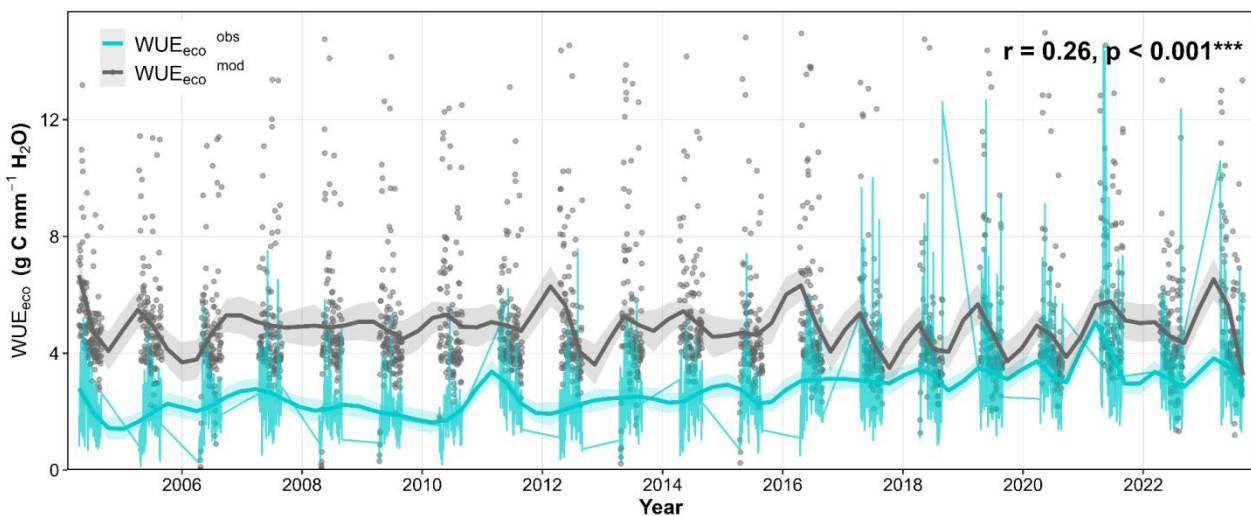
529 3.3 Multi-temporal comparison of observed and modelled WUE_{eco}

531 Daily WUE_{eco} during the growing season exhibited pronounced variability across 2004–2023 (Fig.
532 4). WUE_{eco}^{obs} averaged $2.7 \pm 1.4 \text{ g C mm}^{-1} \text{ H}_2\text{O}$, with episodic peaks to $10\text{--}15 \text{ g C mm}^{-1} \text{ H}_2\text{O}$
533 during mid to late summer periods. WUE_{eco}^{mod} showed higher mean $4.9 \pm 2.7 \text{ g C mm}^{-1} \text{ H}_2\text{O}$.
534 Despite this offset, daily values showed significant positive correlation ($r = 0.26$, $p < 0.001$, $n =$
535 2504), indicating that the model captured aspects of short-term meteorological forcing on carbon–
536 water coupling.

537 LOESS-smoothed trajectories (span = 0.1) revealed distinct temporal patterns between datasets
538 (Fig. 4). During 2004–2014, WUE_{eco}^{obs} remained relatively low ($\sim 2\text{--}3 \text{ g C mm}^{-1} \text{ H}_2\text{O}$) and stable,

539 while WUE_{eco}^{mod} fluctuated around a consistently higher mean ($\sim 4\text{--}5 \text{ g C mm}^{-1} \text{ H}_2\text{O}$), with both
 540 series showing coherent synchronous seasonal oscillations throughout this period. From 2015
 541 onwards, WUE_{eco}^{obs} exhibited a progressive increase, gradually approaching the range of
 542 WUE_{eco}^{mod} , accompanied by intensified within-season variability and more frequent episodic peaks
 543 ($10\text{--}13 \text{ g C mm}^{-1} \text{ H}_2\text{O}$) during mid-to-late summer. Both datasets captured concurrent responses
 544 to notable climatic events, including peaks during 2018 and 2021–2022.

545



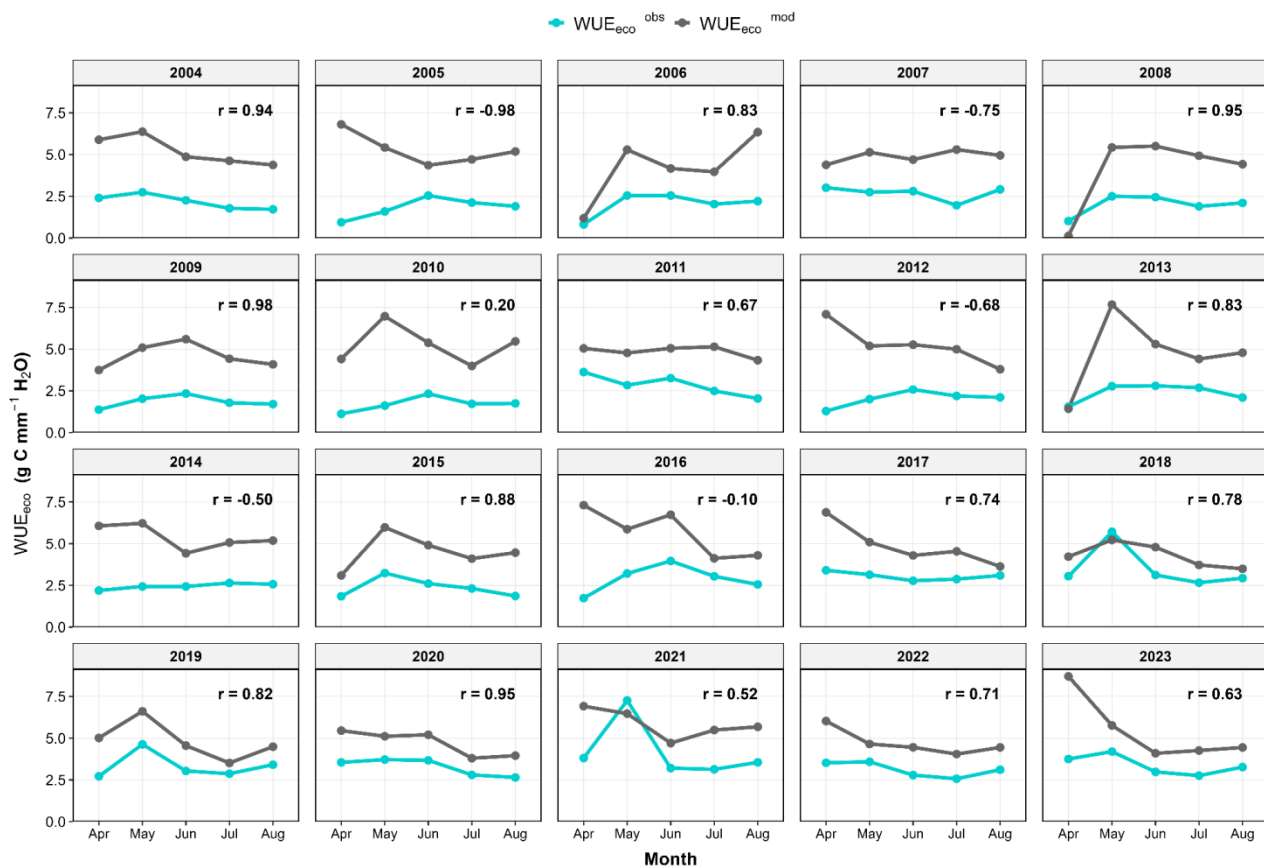
546

547 **Fig. 4** Daily comparison between WUE_{eco}^{obs} (cyan lines) and WUE_{eco}^{mod} (gray points) during the
 548 growing season in a mature *Fagus sylvatica* forest over two decades (2004-2023). Thick smooth
 549 lines represent LOESS trends (span = 0.1) highlighting temporal patterns. Data were filtered using
 550 the 99th percentile of growing-season WUE_{eco} . The Pearson correlation coefficient (r) and
 551 significance level between observed and simulated values are shown (top right). $n = 2504$ daily
 552 observations.

553 At the monthly timescale, WUE_{eco} exhibited higher synchrony within the growing season (April-
 554 August) between WUE_{eco}^{obs} and WUE_{eco}^{mod} across the 2004-2023 study period (Fig. 5). Observed
 555 monthly WUE_{eco}^{obs} averaged $2.7 \pm 0.9 \text{ g C mm}^{-1} \text{ H}_2\text{O}$, while WUE_{eco}^{mod} outputs averaged $4.9 \pm$
 556 $1.2 \text{ g C mm}^{-1} \text{ H}_2\text{O}$, approximately double the observed values.

557 Overall monthly correlation between WUE_{eco}^{obs} and WUE_{eco}^{mod} was statistically significant ($r =$
 558 0.42 , $p < 0.001$, $n = 100$ monthly means), reflecting high interannual variability in model–data
 559 agreement. Annual correlations ranged from $r = -0.98$ to $r = 0.98$, with 15 of 20 years showing
 560 positive correlations.

Temporal analysis revealed progressive improvement in model–data correspondence, with stronger agreement during 2015–2023 ($r = 0.66$, $p < 0.001$, $n = 45$) compared with 2004–2014 ($r = 0.23$, $p = 0.003$, $n = 55$). During years of strong positive correlation, both datasets captured concordant seasonal peaks during May–June and concordant minima during transitional months (April, August), consistent with the phenological constraints of the growing season at this temperate forest site.

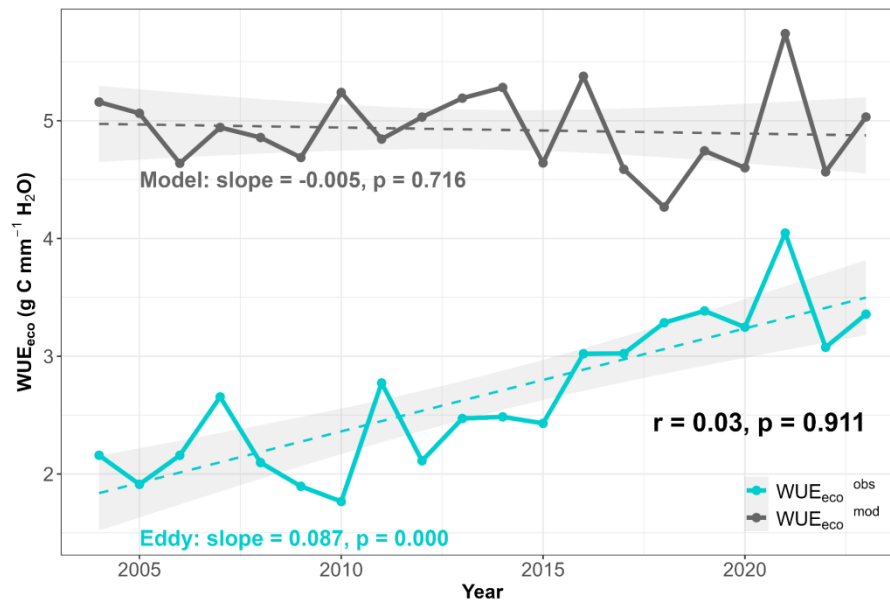


567

Fig. 5 Monthly mean WUE_{eco} during the *F. sylvatica* growing season, 2004–2023, comparing WUE_{eco}^{obs} (cyan line) with WUE_{eco}^{mod} (gray line). Pearson correlation coefficients (r) are shown for each year.

Annual growing-season WUE_{eco} showed substantial differences between WUE_{eco}^{obs} and WUE_{eco}^{mod} (Fig. 6). The 3D-CMCC-FEM model overestimated WUE_{eco}^{mod} (mean: 4.9 ± 0.3 g C mm⁻¹ H₂O) compared WUE_{eco}^{obs} measurements (mean: 2.7 ± 0.6 g C mm⁻¹ H₂O). Temporal dynamics diverged markedly between datasets. WUE_{eco}^{obs} revealed a strong positive trend (slope = 0.083 g C mm⁻¹ H₂O year⁻¹, $p < 0.001$), with WUE_{eco}^{obs} increasing ~55% from 1.8 to 4.0 g C mm⁻¹ H₂O over the study period. Conversely, WUE_{eco}^{mod} exhibited no significant trend (slope = -0.005 , $p = 0.716$), fluctuating within a narrow range (4.3–5.7 g C mm⁻¹ H₂O) around a stable mean (Fig. 6).

Although both time series displayed qualitatively similar interannual patterns from 2015 onwards—including synchronized minima (2017) and maxima (2021-2022), this partial convergence did not translate into overall agreement, with the correlation between annual values remaining weak and non-significant ($r = 0.03$, $p = 0.911$).

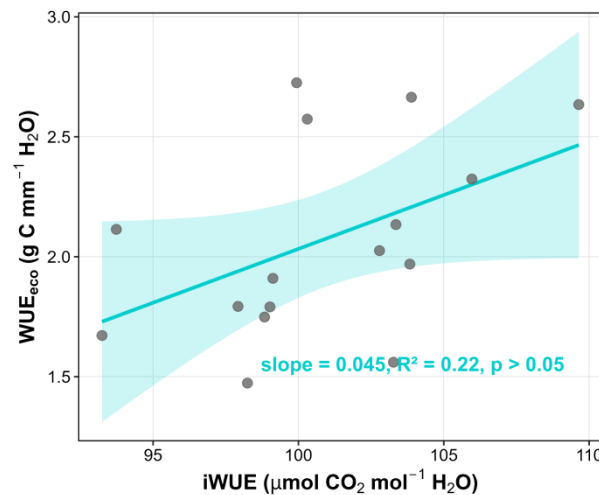


582

Fig. 6 Annual WUE_{eco}^{obs} from eddy covariance observations (cyan) and WUE_{eco}^{mod} (gray) during the growing season of *F. sylvatica* for the period 2004-2023. Dashed lines indicate linear regression trends with shaded areas representing 95% confidence intervals. Trend statistics (slope and p -value) are displayed adjacent to their corresponding trend lines in matching colors. Bold text above the legend shows the Pearson correlation coefficient and significance level between observed and simulated annual values.

Interestingly, the comparison between annually resolved WUE_{eco}^{obs} and WUE_{eco}^{obs} revealed a positive but non-significant relationship ($R^2 = 0.22$, slope = 0.045, $p > 0.05$; Fig. 7), indicating that despite their concurrent increasing tendencies over 2004-2023, the two metrics were not significantly coupled at the annual scale.

593



594

595 **Fig. 7** Relationship between annual $iWUE^{obs}$ derived from tree-ring $\delta^{13}C$ and annual WUE_{eco}^{obs}
 596 derived from EC measurements at the Lägeren site (CH-Lae) over the period 2004-2023 ($n = 20$).
 597 The cyan line represents the linear regression fit with the shaded area indicating the 95% confidence
 598 interval. Regression statistics (slope, R^2 , and p -value) are displayed within the panel.

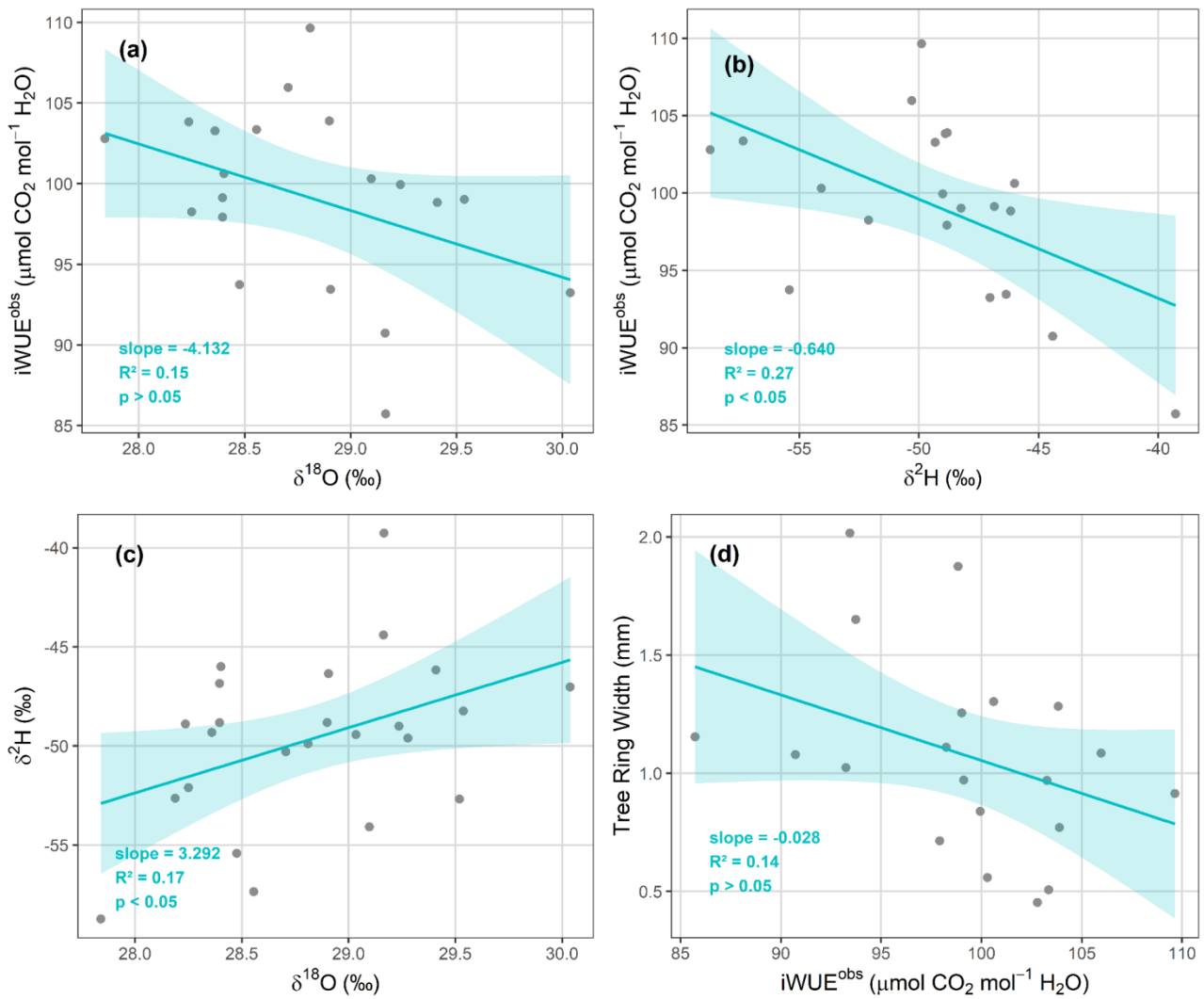
599

600 3.2 Source water isotope controls on $iWUE$ and TRW

601 The relationship between $iWUE^{obs}$ and $\delta^{18}O$ was negative but not statistically significant ($R^2 = 0.15$,
 602 slope = -4.132 , $p > 0.05$; Fig. 8a). In contrast, $iWUE^{obs}$ exhibited a stronger and significant negative
 603 relationship with δ^2H ($R^2 = 0.27$, slope = -0.640 , $p < 0.05$; Fig. 8b). Interestingly, δ^2H and $\delta^{18}O$ in
 604 tree rings were significantly positive related ($R^2 = 0.17$, slope = 3.292 , $p < 0.05$; Fig. 8c).

605 TRW showed a weak negative but non-significant relationship with $iWUE^{obs}$ ($R^2 = 0.14$, slope =
 606 -0.028 , $p > 0.05$; Fig. 8d).

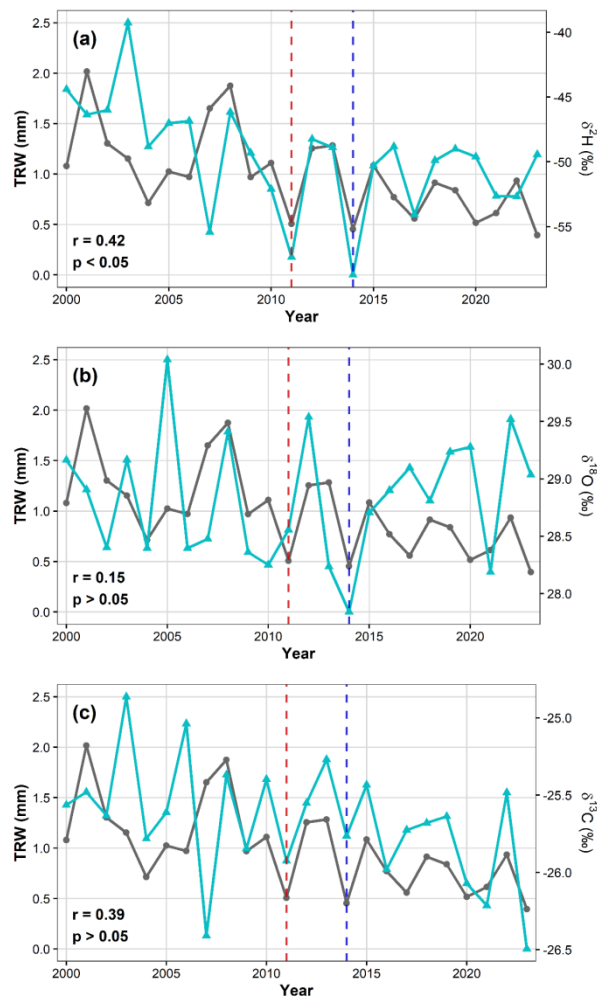
607 Additional insight from triple-isotope relationships revealed a decoupling between carbon and
 608 oxygen isotope signals, as indicated by the weak and non-significant relationship between $\delta^{13}C$ and
 609 $\delta^{18}O$ ($R^2 = 0.02$, $p > 0.05$; Fig. S5a). In contrast, $\delta^{13}C$ exhibited a stronger and significant positive
 610 relationship with δ^2H ($R^2 = 0.29$, $p < 0.01$; Fig. S5b).



611

612 **Fig. 8** Relationships between intrinsic water use efficiency ($iWUE^{obs}$) and isotopic composition of
613 tree rings at CH-Lae for the period 2000-2023. (a) $iWUE^{obs}$ vs. $\delta^{18}\text{O}$ (‰); (b) $iWUE^{obs}$ vs. $\delta^2\text{H}$ (‰);
614 (c) $\delta^2\text{H}$ vs. $\delta^{18}\text{O}$; (d) TRW vs $iWUE^{obs}$. Linear regression lines with 95% confidence intervals
615 (shaded areas in cyan) are shown. Regression parameters (slope, R^2 , p -value) are displayed at
616 bottom left in cyan. Each point represents an individual year ($n = 24$).

617 Interestingly, TRW correlated positively and significantly with $\delta^2\text{H}$ ($r = 0.42$, $p < 0.05$; Fig. 9a),
618 most noticeable during the extreme drought year of 2011 and the coldest year 2014 (Fig. 9a). In
619 contrast, TRW did not correlate significantly with either $\delta^{18}\text{O}$ ($r = 0.15$, $p > 0.05$; Fig. 9b) or $\delta^{13}\text{C}$ (r
620 $= 0.39$, $p > 0.05$; Fig. 9c).



621

622 **Fig. 9** Interannual time series of tree-ring width (TRW, gray lines; right axis) and stable isotopes
623 ratios (cyan lines; right axis) of *F. sylvatica* from 2000-2023: (a) $\delta^2\text{H}$, (b) $\delta^{18}\text{O}$ and (c) $\delta^{13}\text{C}$. The
624 vertical red dashed lines indicate the driest years on record (2011) and the vertical blue dashed line
625 the coldest (2014), both identified from meteorological data at the CH-Lae flux tower.

626 Temporal analysis of $\delta^{18}\text{O}$ and simulated ecosystem stomatal conductance (g_s) showed that the
627 lowest $\delta^{18}\text{O}$ values coincided with the highest simulated g_s during the exceptionally wet summer of
628 2021 (476 mm precipitation; Fig. 2 and Fig. S6). During this year, $\delta^{18}\text{O}$ declined to approximately
629 28.2‰ while g_s reached its maximum ($\sim 0.038 \text{ mol m}^{-2} \text{ s}^{-1}$), consistent with the expected
630 physiological response to abundant water availability.

631

632

633 Discussion

634 4.1 Consistency between modelled and observed $i\text{WUE}$

635 Our multi-proxy analysis revealed a consistent increase in $\delta^{13}\text{C}$ over the last two decades derived
 636 both from tree-ring $\delta^{13}\text{C}$ and simulations of the process-based model 3D-CMCC-FEM. The positive
 637 $\delta^{13}\text{C}$ trend is consistent with the widely documented forest response to rising atmospheric CO_2
 638 across temperate European beech forests, with stronger responses reported in water-limited sites
 639 compared to mesic environments (Frank et al., 2015; Guerrieri et al., 2019; Wang et al., 2025). Our
 640 findings show an approximately 20% increase in $\delta^{13}\text{C}$ over two decades at the Lägeren site,
 641 consistent with these regional patterns (Gharun et al., 2021). The concurrent significant warming
 642 and increasing VPD documented at the site, without a corresponding trend in precipitation, suggest
 643 that the $\delta^{13}\text{C}$ increase reflects both CO_2 fertilization and physiological responses to increasing
 644 atmospheric water demand (Puchi et al., 2024; Scapucci et al., 2025).

645 The significant positive correlation between isotope-derived and modelled $\delta^{13}\text{C}$ indicates that both
 646 approaches consistently captured the interannual dynamics of $\delta^{13}\text{C}$ (Saponaro et al., 2025; 2026).
 647 However, rather than validating one another, they should be regarded as complementary and
 648 independent estimates of leaf-level carbon–water coupling, each integrating physiological processes
 649 over different temporal scales and with different methodological assumptions. The agreement in
 650 temporal trends therefore provides converging evidence that $\delta^{13}\text{C}$ has increased over the last two
 651 decades in this temperate beech forest, while differences in absolute values reflect the inherent
 652 characteristics of each approach rather than inconsistencies in the underlying physiological
 653 response.

654 Observed $\delta^{13}\text{C}$ values ranged between 80 and 110 $\mu\text{mol mol}^{-1}$, consistent with reported values for
 655 temperate forest species under summer conditions (Medlyn et al., 2011; Keenan et al., 2013). In
 656 contrast, modelled $\delta^{13}\text{C}$ averaged approximately 172 $\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$ and exhibited
 657 substantially greater interannual variability. This systematic offset in magnitude is unlikely to
 658 reflect a contradiction between the two approaches, but rather differences in the temporal scales
 659 over which $\delta^{13}\text{C}$ is represented. Whereas isotope-derived $\delta^{13}\text{C}$ integrates physiological responses
 660 throughout the wood-formation period, modelled $\delta^{13}\text{C}$ was calculated from daily simulations
 661 within the optimal intra-annual window identified using DendroTools (21 June–2 August),
 662 corresponding to the period of maximum summer temperatures and atmospheric evaporative
 663 demand. During this period, elevated temperature and VPD simultaneously enhance photosynthetic
 664 assimilation while reducing stomatal conductance, leading to considerably higher instantaneous
 665 $\delta^{13}\text{C}$ values than those preserved in annually integrated tree-ring isotope records (Medlyn et al.,
 666 2017; Grossiord et al., 2020; Novick et al., 2016). Consequently, daily model simulations during
 667 peak summer conditions are expected to produce substantially higher $\delta^{13}\text{C}$ values than annually

integrated tree-ring isotope records, which reflect the cumulative physiological response over the wood-formation period.

Therefore, comparisons between isotope-derived and modelled δ WUE should primarily emphasize temporal variability and long-term trends rather than direct agreement in absolute values. Despite the systematic offset, both approaches consistently identified increasing δ WUE through time, suggesting that they capture common physiological responses to rising atmospheric CO₂ and increasing atmospheric water demand while integrating these responses over different temporal scales. Similar discrepancies have been reported in previous studies comparing isotope-derived physiological proxies with process-based model simulations, highlighting the importance of accounting for temporal aggregation and methodological differences when interpreting δ WUE (De Kauwe et al., 2013; Vitali et al., 2025).

4.2 Decoupling between leaf-level and ecosystem-level water-use efficiency

Despite the strong agreement observed at the leaf scale, our results show that WUE_{eco} derived from EC exhibits markedly different magnitudes and temporal variability compared with δ WUE inferred from tree rings. This mismatch highlights the fundamental scale dependency of WUE metrics, which integrate different processes and spatial domains, temporal scales, and critically species compositions (Li et al., 2022; Grossiord et al., 2020). Whereas δ WUE reflects leaf-level physiological regulation integrated during wood formation, WUE_{eco} represents an emergent property of vegetation–atmosphere interactions across the entire canopy (Medlyn et al., 2017). Critically, the EC signal represents a bulk integrated flux from the entire tower footprint, encompassing all species, soil evaporation, understory vegetation, and canopy interception, making direct comparison with a single-species model inherently challenging (Knauer et al., 2018; Baldocchi, 2003). Consequently, differences between δ WUE and WUE_{eco} should primarily be interpreted in terms of the processes and temporal scales represented by each metric rather than as inconsistencies between the two approaches. Flux-based WUE_{eco} therefore reflects both physiological controls on carbon assimilation and all hydrological processes operating at canopy and soil scales, which may decouple ecosystem-level signals from leaf-level stomatal regulation (Beer et al., 2009; Keenan et al., 2013; Paul-Limoges et al., 2017; Puchi et al., 2025).

The divergence between WUE_{eco}^{obs} and WUE_{eco}^{mod} is primarily attributable to a systematic underestimation of ET by the model (approximately 50% of observed values), rather than to differences in GPP, which remained broadly comparable between the two approaches (Fig. S1). By

700 excluding the water use of co-occurring broadleaf species, evergreen conifers, younger trees, and
 701 understory vegetation, the model omits key contributors to canopy transpiration, interception losses,
 702 and soil water uptake, leading to systematically inflated WUE_{eco}^{mod} values (Anderegg et al., 2019;
 703 Grossiord et al., 2020). Above-canopy EC measurements at Lägeren may additionally be influenced
 704 by advection effects associated with the site's complex topography, and standard friction velocity-
 705 filtering procedures were applied to minimize these effects (Etzold et al., 2010; Aubinet et al.,
 706 2010). An additional and likely source of discrepancy between WUE_{eco}^{obs} and WUE_{eco}^{mod} arises
 707 from differences in species representativeness. The model was parametrized to simulate an old-
 708 growth deciduous stand composed exclusively of mature *F. sylvatica*, whereas EC measurements
 709 integrate fluxes across the entire mixed-species stand, including *F. excelsior* (~20% basal area),
 710 *Acer* sp. (~20%), *A. alba* (~10%), and understory vegetation dominated by *Allium ursinum* in spring
 711 and early summer. The full quantification of these non-transpirational and multi-species water
 712 fluxes is addressed in Section 4.3.

713 The divergent temporal trends, with observations showing a significant positive trend in WUE_{eco}^{obs}
 714 (slope = 0.087, $p < 0.001$) while model simulations remained stable (slope = -0.005, $p = 0.716$),
 715 further support this interpretation. The observed trend likely reflects changes in ecosystem water
 716 use dynamics at CH-Lae, driven by the documented increase in VPD (+36% over the study period)
 717 and differential canopy-level physiological responses across species. In this context, the coupling
 718 between A and g_s is not static but varies with temperature and VPD, and incorporating thermal
 719 sensitivity of stomatal conductance provides important improvements to models of plant carbon-
 720 water exchange under future climate conditions (Chessman et al., 2026). Moreover, increasing
 721 atmospheric demand can partially decouple T from g_s , as T may continue to increase with rising
 722 VPD despite progressive stomatal closure because of the steeper leaf-to-air vapor pressure gradient
 723 (Diao et al., 2024). This nonlinear response implies that ecosystem water loss does not necessarily
 724 scale proportionally with stomatal regulation, thereby contributing to divergence between leaf-level
 725 λWUE and ecosystem-scale WUE_{eco} under high VPD conditions. The presence of evergreen species,
 726 younger trees, and a seasonally dense understory during spring and early summer may have further
 727 amplified these differential responses, as these functional groups exhibit distinct stomatal regulation
 728 strategies compared to mature deciduous *F. sylvatica* (Anderegg et al., 2019; Scapucci et al., 2025;
 729 Shekhar et al., 2025). Since the model simulates only the deciduous old-growth component using a
 730 static stomatal conductance parameterization, it cannot capture these thermally-driven and species-
 731 specific responses, explaining its inability to reproduce the observed positive trend in WUE_{eco}^{obs} .

Further, hydrological partitioning of ET may represent an additional constraint on comparing physiological and ecosystem-scale WUE metrics (Paul-Limoges et al., 2020). While T is directly controlled by stomatal conductance and therefore linked to photosynthesis, ET measured by EC also includes soil evaporation and canopy interception (Knauer et al., 2018; Nelson et al., 2022) and forest management (Saponaro et al., 2026).

T represents between 40% and 80% of total ET in temperate forests, depending on canopy structure, leaf area index, soil moisture availability, and atmospheric demand (e.g., Jasechko et al., 2013; Paul-Limoges et al., 2020). At forest sites with dense canopies and frequent precipitation, such as Lägeren, interception losses can account for 15-30% of total ET, particularly during the full growing season. Furthermore, based on unpublished below-canopy eddy covariance measurements at Lägeren (L. Hörtnagl, pers. comm.), understory ET contributes approximately 10% of the above-canopy flux, a component entirely absent from the single-species model simulation. Taken together, these non-transpirational and understory fluxes represent a substantial and systematic source of ET underestimation in the model, directly explaining the magnitude offset in WUE_{eco} documented in Section 4.2.

Because these water fluxes are not coupled to photosynthetic carbon assimilation by *F. sylvatica*, WUE_{eco} calculated as GPP/ET is expected to be systematically lower than plant-level metrics derived from stomatal processes alone (Knauer et al., 2018; Gong et al., 2022). This mathematical distinction, combined with the structural limitation of the single-species model configuration, provides a comprehensive explanation for the systematic WUE_{eco} offset observed in our study, without invoking differences in carbon assimilation between approaches.

Improving ET partitioning in process-based models therefore represents a critical step toward reconciling leaf-level and ecosystem-scale WUE estimates. Future model developments should incorporate multi-species canopy representations, explicit treatment of evergreen and early-successional components, understory vegetation modules, and improved interception and soil evaporation schemes to more realistically simulate the full water balance of structurally complex mixed-species temperate forests (Knauer et al., 2018; Nelson et al., 2022; Saponaro et al., 2026).

Similar discrepancies between leaf-level and ecosystem-level WUE have been reported in previous studies combining tree-ring isotopes and EC observations (Dekker et al., 2016; Lavergne et al., 2019; Li et al., 2022), but the role of species composition, understory contributions, and ET partitioning in driving these mismatches has only recently been recognized as a central challenge (Wehr et al., 2016; Medlyn et al., 2017).

764

765 **4.3 Temporal scale-dependent dynamics of ecosystem WUE**

766 The comparison between EC and modelled WUE_{eco} further revealed that model-data agreement
 767 improved with temporal aggregation. Daily WUE_{eco} exhibited weak correlations, reflecting the
 768 strong sensitivity of short-term fluxes to rapid fluctuations in solar radiation, VPD, and atmospheric
 769 turbulence. High-frequency meteorological variability can strongly influence stomatal conductance
 770 and canopy transpiration, generating short-term fluctuations that are difficult for ecosystem models
 771 to reproduce accurately (Knauer et al., 2018; Grossiord et al., 2020). Additionally, daily EC
 772 measurements at Lägeren are subject to uncertainty arising from gap-filling procedures, energy
 773 balance closure issues, and advection effects associated with the site's complex topography.
 774 Advection has been estimated to account for up to 36% of net ecosystem productivity at this site,
 775 and standard u-filtering was applied to minimize these effects, although residual advective losses
 776 may still influence daily flux estimates (Baldocchi, 2003; Etzold et al., 2010; Aubinet et al., 2012;
 777 Medlyn et al., 2017).

778 These results are consistent with recent multi-scale studies showing that WUE depends on the
 779 biological scale at which it is evaluated. Rambal et al. (2025) similarly reported contrasting drought
 780 responses between $iWUE$ and WUE_{eco} . Our results extend this perspective by integrating tree-ring
 781 isotopes, eddy covariance observations, and process-based modelling to provide a mechanistic
 782 interpretation of these scale-dependent differences.

783 When aggregated to monthly timescales, the agreement between observed and simulated WUE_{eco}
 784 improved substantially, indicating that seasonal patterns of carbon-water coupling are more reliably
 785 reproduced by the model than short-term variability (Ito & Inatomi 2012). This improvement
 786 reflects the fact that monthly aggregation averages out high-frequency meteorological noise and
 787 measurement errors, allowing the underlying physiological responses to temperature, radiation, and
 788 soil moisture cycles to emerge more clearly (Medlyn et al., 2017; Lavergne et al., 2019).

789 At this temporal scale, the model's representation of phenological development, seasonal
 790 acclimation of photosynthetic capacity, and progressive soil water depletion appears adequate to
 791 capture the dominant controls on ecosystem carbon-water coupling. The synchronous responses to
 792 climatic events, such as the 2018 drought (Gharun et al., 2020) and the 2021 wet year, further
 793 demonstrate that the model reproduces key aspects of interannual variability when integrated over
 794 monthly periods (Reyer et al., 2020; Collalti et al., 2016).

795

796 **4.4 Disentangling physiological and hydrological signals driving WUE trends across scales**

797 The triple isotope analysis ($\delta^{13}\text{C}$, $\delta^{18}\text{O}$, $\delta^2\text{H}$) of tree rings provided complementary insights into the
 798 mechanisms underlying long-term changes in $i\text{WUE}$ and WUE_{eco} trends at the Lägeren site. Rather
 799 than reflecting a single physiological process, the three isotopes integrate different components of
 800 the soil–plant–atmosphere continuum. Consistent with previous studies (Scheidegger et al., 2000;
 801 Gessler et al., 2014; Vitali et al., 2022), our results suggest that carbon and water-related isotope
 802 signals become increasingly decoupled under mesic conditions, emphasizing that long-term $i\text{WUE}$
 803 trends cannot be interpreted solely in terms of stomatal regulation but must also account for
 804 hydrological controls associated with source-water uptake and transport.

805 The isotope evidence provides important mechanistic insights into the processes underlying the
 806 observed increase in $i\text{WUE}$ (Gessler et al., 2014; Saurer & Voelker, 2022). If the long-term increase
 807 had been driven primarily by progressive stomatal closure, increasing evaporative enrichment of
 808 leaf water would be expected to produce higher $\delta^{18}\text{O}$ values in tree-ring cellulose (Scheidegger et
 809 al., 2000; Barbour et al., 2004). However, neither $\delta^{18}\text{O}$ nor $\delta^2\text{H}$ exhibited patterns consistent with
 810 progressive isotopic enrichment, indicating that sustained reductions in stomatal conductance were
 811 unlikely to be the dominant mechanism underlying the observed increase in $i\text{WUE}$. Instead, our
 812 results suggest that the increase in $i\text{WUE}$ was driven predominantly by enhanced photosynthetic
 813 carbon assimilation (A), or by a combination of increased assimilation and relatively modest
 814 adjustments in stomatal conductance, consistent with the conceptual dual-isotope framework
 815 proposed by Scheidegger et al. (2000).

816 The positive and significant relationship between $\delta^2\text{H}$ and $\delta^{18}\text{O}$ in tree rings of *F. sylvatica* ($R^2 =$
 817 0.27) is stronger than reported from longer European chronologies (Vitali et al., 2022), suggesting
 818 that both isotopes were governed by similar hydrological processes at the LAE site. Moreover, this
 819 coupling indicates that trees remained well acclimatized to the prevailing environmental conditions
 820 during the study period (Vitali et al., 2024). Although $\delta^2\text{H}$ is generally considered less responsive
 821 than $\delta^{18}\text{O}$ to evaporative enrichment associated with atmospheric demand it can also be influenced
 822 by post-photosynthetic metabolic fractionation under drought or physiological stress, leading to a
 823 decoupling between $\delta^2\text{H}$ and $\delta^{18}\text{O}$ signals (Cormier et al., 2018; Lehmann et al., 2021). However,
 824 the significant positive relationship observed in our study provides little evidence that such
 825 metabolic processes dominated $\delta^2\text{H}$ variability during the study period. Instead, the coupled
 826 behaviour of $\delta^2\text{H}$ and $\delta^{18}\text{O}$ suggests that both isotopes were primarily controlled by hydrological

827 processes, including source-water isotopic composition and leaf-water enrichment during tree-ring
828 formation. This interpretation is consistent with Brinkmann et al. (2018), who demonstrated that
829 xylem water integrates both recent precipitation and older soil water stored over several months,
830 with winter precipitation contributing substantially to transpiration during the subsequent growing
831 season in *F. sylvatica* at CH-Lae. Consequently, interannual variability in precipitation inputs, soil
832 water residence time, and rooting depth can exert a dominant control on the isotopic composition of
833 source water before isotopic signals are modified by leaf-level physiological processes.

834 Consequently, the negative relationships between $\delta^{13}\text{C}$ -derived δWUE and both $\delta^{18}\text{O}$ and $\delta^2\text{H}$
835 suggest that leaf physiological processes are closely linked to source-water origin and uptake
836 dynamics (e.g., recent precipitation versus shallow or deeper soil water) at the CH-Lae site. During
837 relatively dry conditions, enhanced reliance on deeper soil water reserves, which are generally more
838 isotopically depleted because they integrate older precipitation inputs, may simultaneously increase
839 δWUE while producing lower $\delta^{18}\text{O}$ and $\delta^2\text{H}$ values in tree rings (Brinkmann et al., 2018). These
840 findings indicate that interannual variability in source-water isotopic composition largely overprints
841 physiological signals in both isotopes under the mesic conditions prevailing at Lägeren.

842 Nevertheless, this dominance of hydrological controls does not exclude a physiological contribution
843 to $\delta^2\text{H}$. Unlike $\delta^{18}\text{O}$, hydrogen isotopes are increasingly recognized as integrating both hydrological
844 processes and post-photosynthetic metabolism, including carbohydrate remobilization and carbon
845 allocation (Lehmann et al., 2021; Vitali et al., 2022). Their Europe-wide synthesis demonstrated
846 that $\delta^2\text{H}$ is a weaker climatic proxy than $\delta^{13}\text{C}$ or $\delta^{18}\text{O}$ because it contains a substantial physiological
847 component related to metabolic fractionation. Therefore, although source-water variability appears
848 to dominate $\delta^2\text{H}$ at the CH-Lae site under average conditions, physiological processes may become
849 increasingly important during years characterized by exceptional drought or other environmental
850 stress, when changes in carbon allocation and the utilization of stored assimilates are expected to
851 contribute more strongly to the hydrogen isotope signal. This mechanism could explain deviations
852 from the otherwise strong hydrological coupling between $\delta^2\text{H}$ and $\delta^{18}\text{O}$ observed during extreme
853 years (Holloway-Phillips et al., 2026).

854 Moreover, assuming that $\delta^{18}\text{O}$ in tree rings primarily reflects source-water isotopic composition
855 rather than evaporative enrichment, our results suggest a relatively low sensitivity of $\delta^{18}\text{O}$ to leaf-
856 water enrichment and stomatal regulation at CH-Lae. This interpretation is supported by the
857 negative relationship between $\delta^{18}\text{O}$ and model-simulated g_s , with appreciable sensitivity observed
858 only during the exceptionally wet year of 2021. Likewise, the three driest growing seasons (2011,
859 2015 and 2018) produced only modest changes in both $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ (Fig. 9), indicating that severe

summer drought did not induce the pronounced isotopic enrichment expected under sustained stomatal limitation. This muted response is consistent with the interpretation that mature *Fagus sylvatica* buffered drought through continued access to relatively stable soil water sources, thereby maintaining leaf water status and reducing evaporative enrichment despite high atmospheric demand. Consequently, the weak relationship between $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ limits the direct application of the dual-isotope conceptual framework for separating the relative contributions of photosynthetic assimilation (A) and stomatal conductance (g_s) at this site. (Scheidegger et al., 2000; Siegwolf et al., 2023). Instead, the limited sensitivity of $\delta^{18}\text{O}$ to g_s suggests that the observed increase in $i\text{WUE}$ over the last two decades was driven primarily by enhanced photosynthetic carbon assimilation rather than sustained stomatal closure. This interpretation is directly supported by the significant positive trend in GPP observed over the study period, in both eddy covariance (slope = 18.98, $p < 0.001$) and model estimates (slope = 24.20, $p < 0.001$), with strong correlation between the two approaches ($r = 0.63$, $p < 0.01$; Fig. S1). The concurrent increase in GPP and $i\text{WUE}$ suggests that enhanced CO_2 fertilization sustained carbon assimilation capacity, while the isotopic composition of source water, rather than stomatal regulation, remained the dominant control on $\delta^{18}\text{O}$ and $\delta^2\text{H}$ variability at this site.

Under high soil moisture conditions, plants maintain open stomata to maximize carbon assimilation without risk of hydraulic stress, resulting in elevated transpiration rates. High transpiration reduces the residence time of water in leaves, thereby limiting evaporative $\delta^{18}\text{O}$ enrichment and producing lower (more negative) $\delta^{18}\text{O}$ values in cellulose (Barbour et al., 2004; Gessler et al., 2014; Cernusak et al., 2016). Conversely, during water-limited periods, stomatal closure reduces transpiration, prolonging leaf water residence time and enhancing evaporative enrichment, which results in higher $\delta^{18}\text{O}$ values in tree-ring cellulose (Barbour et al., 2004; 2007). Overall, the combined isotope, eddy covariance, and modelling evidence indicates that carbon–water interactions at the Lägeren forest are governed by both physiological regulation and hydrological buffering. While isotope-derived $i\text{WUE}$ and process-based simulations consistently captured long-term physiological adjustments, ecosystem-scale WUE reflected additional controls associated with ET partitioning, species composition, and ecosystem structure. Together, these findings demonstrate that interpreting forest water-use efficiency requires explicit consideration of the spatial and temporal scales represented by each measurement approach.

5. Conclusions

WUE is widely used to assess forest responses to climate change, yet its interpretation depends strongly on the spatial and temporal scales represented by different measurement approaches. Rather than providing alternative estimates of the same process, tree-ring stable isotopes, eddy covariance observations, and process-based models each capture complementary aspects of carbon–water interactions. Our study demonstrates that integrating these approaches provides a robust mechanistic framework for interpreting WUE across biological scales and for improving predictions of forest responses to increasing atmospheric CO₂, rising atmospheric water demand, and future climatic extremes. Within this framework, the main findings of our study are:

- The strong agreement between $iWUE^{obs}$ and $iWUE^{mod}$ ($r = 0.61$, $p < 0.05$) is a direct consequence of the species-specific consistency between approaches: both the isotopic chronology and the 3D-CMCC-FEM model simulations represent exclusively the physiology of mature *F. sylvatica*, confirming that the model successfully captures leaf-level physiological coupling between photosynthetic assimilation and stomatal conductance for this species.
- The multi-isotope evidence demonstrates that the observed ~20% increase in $iWUE$ over the study period is predominantly driven by enhanced photosynthetic assimilation (A) under rising CO₂, rather than by progressive stomatal closure, as source water dynamics rather than evaporative enrichment dominated the $\delta^{18}O$ and δ^2H signals at Lägeren.
- The divergence between WUE_{eco}^{obs} and WUE_{eco}^{mod} is primarily attributable to the systematic underestimation of ET by the model (~50% of observed values), resulting from its single-species old-growth deciduous configuration that excludes the water use of co-occurring broadleaf species, evergreen conifers, younger trees, and understory vegetation, the latter contributing approximately 10% of above-canopy ET at Lägeren.
- Model–data agreement improved substantially with temporal aggregation, indicating that the model reproduces seasonal and interannual patterns more reliably than short-term variability. The improvement from daily to monthly timescales highlights the strong influence of high-frequency meteorological variability, atmospheric turbulence, and flux measurement uncertainty on ecosystem-scale WUE.
- The absence of a significant relationship between intrinsic and ecosystem WUE demonstrates that these metrics should not be interpreted interchangeably. Intrinsic WUE reflects leaf-level physiological regulation, whereas ecosystem WUE integrates additional hydrological, structural, and community-level processes. Consequently, long-term reconstructions of $iWUE$ based on tree-ring $\delta^{13}C$ cannot be directly extrapolated to

ecosystem-scale carbon–water exchange without considering these scale-dependent processes

Overall, this study presents, to our knowledge, the first mechanistic framework integrating tree-ring stable isotopes, eddy covariance observations and process-based ecosystem modelling to disentangle WUE from leaves to ecosystems. This integrative approach moves beyond simple comparisons among methodologies by identifying the biological and hydrological processes responsible for scale-dependent carbon–water coupling, thereby providing a new framework for improving predictions of forest responses to global change.

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Competing interests

None declared.

Author contributions

PFP: Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Data curation, Conceptualization, Funding acquisition; DD: Writing – review & editing, Validation, Methodology, Formal analysis; DC: Writing – review & editing; EV: Methodology, Data curation; MML: Methodology, Formal analysis, Conceptualization, review & editing; MS: review & editing, Investigation, Formal analysis, Project administration, Funding acquisition; SK: review & editing, Formal analysis, Data curation, Conceptualization; LH: review & editing, Methodology, Formal analysis, Data curation; AC: Writing – review & editing, Writing – original draft, Project administration, Funding acquisition.

955

956 **Data availability**

957 **Tree-ring chronology and triple isotopes data.** Data will be made available on request to the
958 corresponding author.

959 **Modelled data are deposited and publicly available in Zenodo:**
960 <https://doi.org/10.5281/zenodo.20928067>

961

962 **Eddy covariance (EC) observational data can be accessed from the following public**
963 **repositories:** <https://www.swissfluxnet.ethz.ch/index.php/sites/site-info-ch-lae/>

964 The source code used in this study is freely available at the same Zenodo repository as the modelled
965 data.

966

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