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Impacts of elevated CO_2 levels and temperature on photosynthesis and stomatal closure along an altitudinal gradient are counteracted by the rising atmospheric vapor pressure deficit

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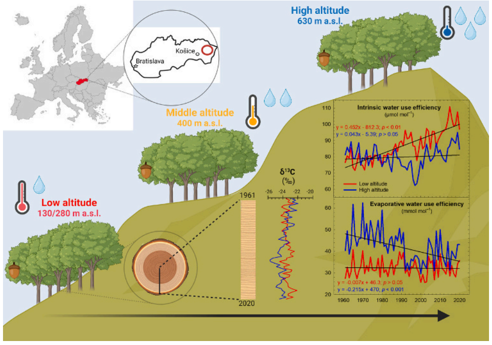
HIGHLIGHTS

- Carbon isotopes assess water use efficiency (WUE) in oaks along altitudinal gradient.
- High sensitivity allows to distinguish even among trees of small mountain ranges.
- Trees at low altitudes show increased stomata-based WUE over the past 60 years.
- Increasing vapor-pressure deficit (VPD) offsets CO_2 and temperature benefits on WUE.
- Atmospheric VPD correction is necessary when calculating WUE at large scales.

GRAPHICAL ABSTRACT



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ABSTRACT

The efficiency of water use in plants, a critical ecophysiological parameter closely related to water and carbon cycles, is essential for understanding the interactions between plants and their environment. This study investigates the effects of ongoing climate change and increasing atmospheric CO_2 concentration on intrinsic (stomata-based; iWUE) and evaporative (transpiration-based; eWUE) water use efficiency in oak trees along a naturally small altitudinal gradient (130–630 m a.s.l.) of Vihorlat Mountains (eastern Slovakia, Central Europe). To assess changes in iWUE and eWUE values over the past 60 years (1961–2020), stable carbon isotope ratios in latewood cellulose ($\delta^{13}C_{cell}$) of annually resolved tree rings were analyzed. Such an approach was sensitive enough to distinguish tree responses to growth environments at different altitudes. Our findings revealed a rising trend in iWUE, particularly in oak trees at low and middle altitudes. However, this increase was negligible at high altitudes. Warmer and drier conditions at lower altitudes likely led to significant stomatal closure and enhanced

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efficiency in photosynthetic CO_2 uptake due to rising CO_2 concentration. Conversely, the increasing intracellular-to-ambient CO_2 ratio (Ci/Ca) at higher altitudes indicated lower efficiency in photosynthetic CO_2 uptake. In contrast to iWUE, eWUE showed no increasing trends over the last 60 years. This suggests that the positive impacts of elevated CO_2 concentrations and temperature on photosynthesis and stomatal closure are counteracted by the rising atmospheric vapor pressure deficit (VPD). These differences underscore the importance of the correct interpretation of stomata-based and transpiration-based WUEs and highlight the necessity of atmospheric VPD correction when applying tree-ring δ^{13}C-derived WUE at ecosystem and global levels.

1. Introduction

Forests play a vital role in carbon sequestration, constituting up to 90 % of terrestrial carbon biomass (Pan et al., 2011). Forest ecosystems act as significant carbon sinks, mitigating climate change intensified by fossil fuel burning and rising atmospheric CO_2 concentrations. Predicting how future growth conditions impact tree physiology, especially concerning water and carbon cycles, is paramount. A useful strategy for understanding climate change effects on plants and ecosystems involves comparing populations of the same species across diverse climates along altitudinal and/or latitudinal gradients (De Frenne et al., 2013; Leroy et al., 2020). Altitudinal gradients, characterized by rapid changes in temperature, humidity, precipitation, and sun radiation within small geographic areas (Körner, 2007), offer insights into plant growth responses to varied climate conditions (Jump et al., 2009). These factors not only influence the growing period's onset and duration, but also directly impact plant physiological processes, affecting carbon uptake and plant water status (Bošel'a et al., 2014; Bresson et al., 2011; King et al., 2013; Rajsnerová et al., 2015; Vitasse et al., 2009).

Previous studies have shown decreasing growth rate and tree-ring width (TRW) with increasing altitude (e.g., Hartl et al., 2022; Paulsen et al., 2000; Peñuelas et al., 2008; Ponocná et al., 2016). It is likely due to differences in summer temperatures between low and high altitudes, when TRW is often constrained by low summer temperatures in cold high altitudinal sites (King et al., 2013; Splechtna et al., 2000). Gas exchange studies on the leaf level have further revealed that plants at lower altitudes typically exhibit lower transpiration rates and stomatal conductance compared to their high-altitudinal counterparts (Bresson et al., 2009, 2011; McDowell et al., 2008; Rajsnerová et al., 2015). This reduction in stomatal conductance does not hinder enhanced CO_2 assimilation, likely due to significantly higher RuBisCO (Ribulose-1,5-Bisphosphate Carboxylase/Oxygenase) content in low-altitudinal trees (Mediavilla et al., 2016; Rajsnerová et al., 2015). Such a disparity results in enhanced water use efficiency, calculated as the carbon assimilated per unit of water lost, in low-altitudinal plants, at least in the short term. This parameter, rooted in plant physiology, plays a pivotal role in Earth's interconnected carbon and water cycles and holds great potential in indicating plant adaptability to changing growth conditions (Cheng et al., 2017; El Masri et al., 2019).

Increasing trends in water use efficiency along altitudinal gradients were also confirmed at the level of whole plants using the technique of isotope discrimination (Peñuelas et al., 2008; Wu et al., 2015). In particular, the stable carbon isotope ratio δ^{13}C ($^{13}C/^{12}C$) of tree rings has been found to be a useful indicator of annual changes in intrinsic water use efficiency, iWUE (Adams et al., 2020; Dorado-Liñán et al., 2020; Frank et al., 2015; Oulehle et al., 2023; Waterhouse et al., 2004), defined as the ratio between the rate of CO_2 assimilation and the stomatal conductance to water vapor (Osmond et al., 1980; Ehleringer et al., 1993; Saurer and Voelker, 2022). Higher representation of the ^{13}C isotope yields less negative δ^{13}C values, indicating higher iWUE. This increase may stem from improved CO_2 assimilation (Giammarchi et al., 2017; Guerrieri et al., 2019; Mathias and Thomas, 2021), reduced stomatal conductance (Andreu-Hayles et al., 2011; Lévesque et al., 2014; Saurer et al., 2014; Waterhouse et al., 2004; Yang et al., 2021) or both. While stomata tend to close under conditions of water scarcity or high atmospheric vapor pressure deficit (Grossiord et al., 2020), photosynthetic CO_2 assimilation increases with higher light intensity, nutrient availability, temperature, and CO_2 concentration (Kubiske and Pregitzer, 1996; Moore et al., 2021). Therefore, interpreting annual changes in iWUE over decades requires careful consideration of changes in atmospheric CO_2 concentration and its isotopic composition, along with changes in other abiotic factors such as soil acidification affecting calcium availability, which has a major effect on stomata functioning (Oulehle et al., 2023). Among other nutrients, increased availability of nitrogen and phosphorus has been shown to stimulate iWUE, primarily due to an enhancement in the rate of photosynthetic CO_2 assimilation (Dijkstra et al., 2016).

However, iWUE might not accurately reflect plant water use efficiency because plant water status primarily depends on the total volume of transpired water rather than stomatal conductance (Farquhar et al., 1989a, 1989b; Ehleringer et al., 1993; Saurer and Voelker, 2022). The total transpiration rate is primarily determined by the evaporative surface size (leaf area) and numerous environmental factors that influence stomatal openness, boundary layer thickness on the leaf surface, and atmospheric conditions. Among these factors, atmospheric vapor pressure deficit (VPD - the difference between the atmospheric water amount at a given temperature and the water amount the atmosphere could hold at saturation) plays a significant role (Cheng et al., 2017; Fletcher et al., 2007; Kolb and Stone, 2000; Treydte et al., 2024). Transpiration rates increase in dry atmospheres but significantly decrease in wet atmospheres. However, iWUE does not capture this VPD-driven phenomenon (Ehleringer et al., 1993). Therefore, the evaporative water use efficiency (eWUE), accounting for atmospheric humidity, has been introduced (Eq. (5) in this study; reviewed in Seibt et al., 2008 or Saurer and Voelker, 2022).

This study evaluates both iWUE and eWUE in oak species growing along their natural altitudinal gradient (130–630 m a.s.l.) in the western regions of Central Europe over the last 60 years (1961–2020). The research is based on the stable carbon isotope composition of tree-ring cellulose from two oak species (*Quercus robur* L. and *Quercus petraea* (Matt.) Liebl.) prevalent in Europe (Eaton et al., 2016). The study area, located in the Vihorlat Mountains (eastern part of Slovakia), is unique due to the presence of numerous old trees of the same species, all with the same slope exposure and similar soil conditions (Bosel'a et al., 2021; Hartl-Meier et al., 2015). The primary goal was to test the hypothesis that climate change, characterized by rising atmospheric CO_2 and temperature levels and significant drought periods, leads to a gradual increase in tree water use efficiency over the years. More specifically, we hypothesized that (i) water use efficiency increases differently at different altitudes, assuming (ii) a pronounced effect on iWUE, especially at lower altitudes, but (iii) a minimal effect on eWUE when considering atmospheric humidity.

2. Material and methods

2.1. Site description

The forest under investigation is situated on the southwestern slope of the Vihorlat Mountains, a volcanic massif spanning eastern Slovakia and western Ukraine in Central Europe. Oak, beech, hornbeam, and pine are the most common naturally growing tree species in this area. The region has a continental climate with hot summers, cold winters, and moderate amounts of precipitation. The growing season, occurring from April to August, is characterized by mean temperatures and precipitation amounts ranging between 11.2 and 19.8 °C and between 168 and 721 mm, respectively. However, in the past two decades, there has been an increase in the frequency of dry seasons (total seasonal precipitation below 345 mm; first quartile for the investigated period 1961–2020) and hot seasons (mean seasonal temperatures above 15.5 °C; third quartile), accompanied by increased interannual variability in climate parameters (Supplementary Tables S1 and S2).

In this study, primarily sessile oak trees (*Quercus petraea*) were sampled at low altitude (LA; 280 m a.s.l.), middle altitude (MA; 400 m a.s.l.), and high altitude (HA; 630 m a.s.l.). Based on the available professional forestry maps, these altitudes represent the natural occurrence range for sessile oak trees in the given region. Additionally, we sampled English oak trees (*Q. robur*) at the altitude of 130 m a.s.l. as a supplementary material. Sampling sites are located approximately 90 km NE of Košice (SK), 240 km SW of Lviv (UA), 330 km SE of Krakow (PL), and 350 km NE of Budapest (HU) (see the map in Graphical Abstract and Supplementary Fig. S1). The specific coordinates for each site are provided in Table 1.

Daily minimum, mean and maximum temperatures and air humidity from ~300 climatological stations and daily precipitation totals from 1100 rain-gauge stations of the Czech Hydrometeorological Institute (CHMI) and Slovak Hydrometeorological Institute (SHMI) were used as source of climate data. The vapor pressure deficit (VPD) for each site was estimated using a variation of Tetens' (1930) formula following Allen et al. (1998). The location of the nearest SHMI stations is shown in Supplementary Fig. S1. The entire dataset of CHMI and SHMI data was then used to estimate daily values for 0.5-km × 0.5-km grids for the entire 1961–2020 period. Daily data are interpolated by regression through kriging, which uses geographical coordinates, altitude, and other terrain characteristics as predictors. Within the CHMI and SHMI domain, the average minimum distance between two neighboring stations is approximately 22 km for elements measured at climatological stations and <10 km for elements measured at precipitation stations. For each sampling site, the most corresponding 0.5-km grid was used in terms of distance and altitude.

Climate data for the vegetation period (April–August) from 1961 to 2020 are shown in Fig. 1. While the mean air temperatures during this period (15.5, 14.7, and 13.5 °C) and the vapor pressure deficit (0.57, 0.52, and 0.46 kPa) decrease with increasing altitude, the mean relative air humidity (67.8, 69.1, and 70.8 %) and the precipitation (393, 418, and 458 mm) increase with altitude. These trends were confirmed at the altitude of 130 m a.s.l., where temperature, VPD, relative air humidity, and precipitation amount to 16.6 °C, 0.65 kPa, 65.8 %, and 345 mm, respectively (Supplementary Fig. S2 and Supplementary Table S1).

2.2. Sample collection

First, 15 representative trees were selected at each site and one core per tree was taken at breast height (1.3 m) using an increment borer (Haglöf Company Group, Långsele, Sweden) with an inner diameter of 5 mm (Gut et al., 2019). Tree-ring width (TRW) series were measured on all samples using a VIAS TimeTable (SCIEM, Brunn, Austria) with an accuracy of 0.01 mm. The obtained TRW series were cross-dated using PAST4 software (SCIEM, Brunn, Austria), corrected for missing and false rings, and visually compared (Rybníček et al., 2010). The site TRW chronologies were calculated using bi-weight robust means. The most recent version of the Slovakia oak TRW composite chronology (Prokop et al., 2016) was used for verification and absolute dating of the site TRW chronologies. Mean values of inter-site correlations of TRW series (*R*bar) ranged between 0.32 and 0.52 over the period 1961–2020 ($p < 0.01$).

Based on the TRW analysis, six trees from each site were subsequently selected for the analysis of stable carbon isotopes in non-pooled tree rings. Thus, TRW series was used for absolute dating of the samples and selection of representative trees for isotopic analysis. The set of six trees represents a good compromise between the loss of explained variance and the cost of isotopic analysis (Rybníček et al., 2021).

2.3. Cellulose extraction and $\delta^{13}C_{cell}$ determination

The absolutely dated annual increments of individual trees from the period 1961–2020 were cut into small pieces with a scalpel under a stereomicroscope and packed into F57 Teflon bags (Ankom Technology, Macedon, NY, USA) (Rybníček et al., 2021). Samples included particularly latewood, considered a robust archive of stable carbon isotopes reflecting the climate of the current year, but samples from the most narrow tree rings could contain a greater proportion of earlywood. A previous study on oaks showed that the relative proportion of latewood in a sample does not have a statistically significant effect on the ratio of stable carbon isotopes (Torbenson et al., 2023), although the carbon isotope composition of the earlywood could be affected by the remobilization of carbohydrate reserves from the previous year (Hill et al., 1995).

The modified Jayme-Wise isolation method was used to extract the α-cellulose from each tree ring (Boettger et al., 2007). Teflon filter bags were washed twice in a 5 % NaOH solution at 60 °C for 2 h, followed by another wash in a 7 % $NaClO_2$ solution at 60 °C for an additional 30 h. To keep the pH at 4–5, acetic acid (99.8 %) was added to the solution. Teflon bags containing extracted α-cellulose were washed and then rinsed three times in hot distilled water (90 °C). Such an extraction procedure has been tested and proven sufficient for extracting cellulose from the wood of living oaks that have not been contaminated or chemically treated (Urban et al., 2021). The samples were subsequently dried at 50 °C for 24 h, locked in Eppendorf microtubes, and stored in the dark at 21 °C until analysis.

For the determination of $\delta^{13}C_{cell}$, homogenized α-cellulose (approximately 1.0 mg) was weighed into tin capsules (Elementar Analysensysteme, Langenselbold, Germany) and combusted at 960 °C to CO_2 using an elemental analyzer varioPYRO cube (Elementar Analysensysteme, Germany). The abundance of ^{12}C and ^{13}C stable isotopes in CO_2 was determined by a continuous flow isotope ratio mass spectrometer ISOPRIME100 (Isoprime, Manchester, UK). The system was

Table 1
Summary information for sites and trees used for the isotope measurement.
Abbreviations: HA (high altitude), MA (middle altitude), LA (low altitude), and DBH (diameter at breast height of all analyzed trees).

Site	Altitude (m a.s.l.)	Species	Mean age (years) *(min–max)*	Mean DBH (cm) *(min–max)*	GPS coordinates
HA	630	*Q. petraea*	150 *(145–154)*	45.8 *(39.0–58.3)*	48.8403189N 22.2440050E
MA	400–430	*Q. petraea*	125.3 *(120–130)*	46.6 *(38.4–62.0)*	48.8165794N 22.2371753E
LA	280	*Q. petraea*	124.2 *(113–134)*	49.0 *(42.6–57.2)*	48.8013189N 22.2407319E
Supplemental LA site	130–135	*Q. robur*	121.2 *(109–141)*	68.8 *(64.4–71.5)*	48.7635489N 22.1879789E

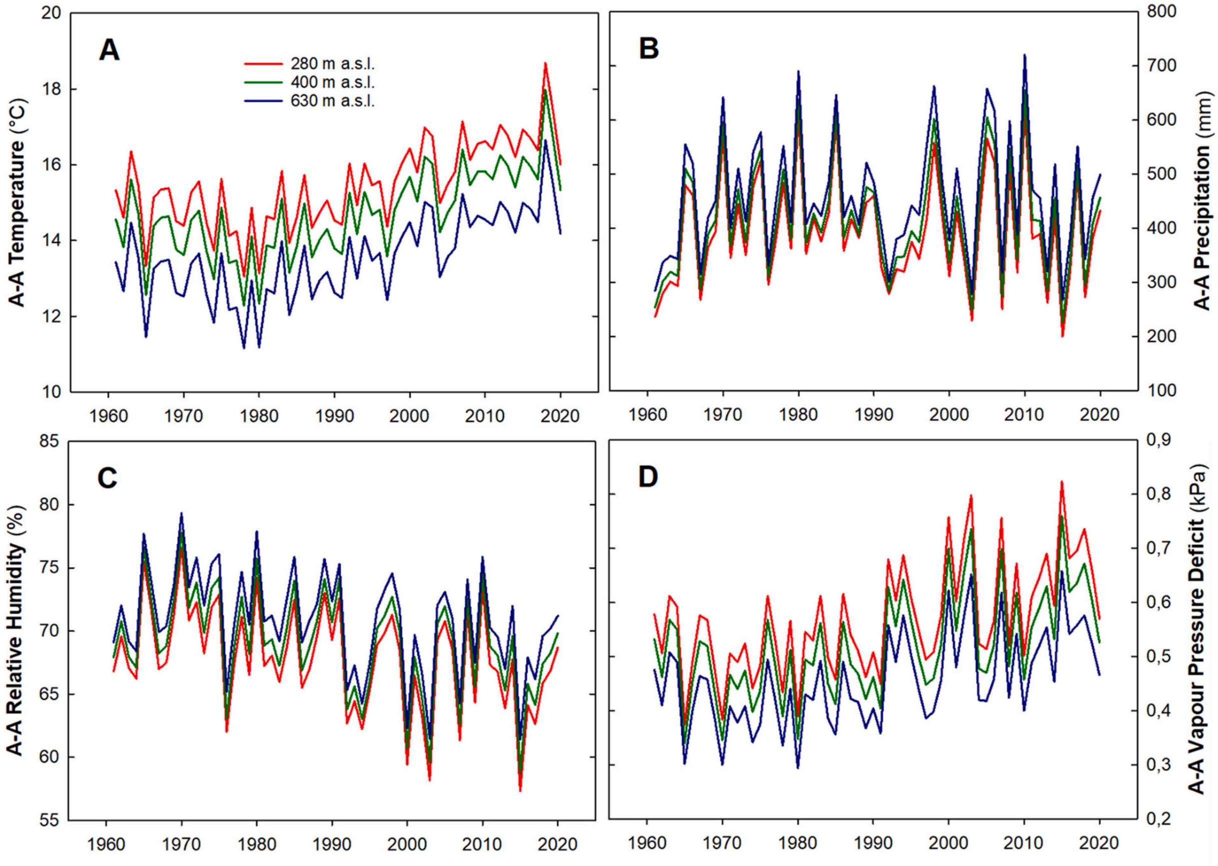

Fig. 1. Climate data over the vegetation season (April–August) of the investigated 60-year-long period (1961–2020) at three altitudes: low altitude (LA; 280 m a.s.l.), middle altitude (MA; 400 m a.s.l.), high altitude (HA; 630 m a.s.l.). (A) Mean air temperature, (B) total precipitation, (C) mean relative air humidity, and (D) mean atmospheric vapor pressure deficit. See Supplementary Table S1 for details.

internally calibrated using certified reference materials of known isotopic ratios from the International Atomic Energy Agency (IAEA, Vienna, Austria) and the US Geological Survey (USGS, USA): caffeine (IAEA-600) and graphite (USGS24). The $^{13}C/^{12}C$ ratio in tree-ring cellulose ($\delta^{13}C_{cell}$ in ‰) was related to the Vienna Pee Dee Belemnite (VPDB) standard according to the formula:

$$\delta^{13}C_{cell} = \left(\frac{R_{cell}}{R_{VPDB}} - 1\right) \times 1000 \tag{1}$$

where R is the ratio of the heavy to light isotope ($^{13}C/^{12}C$) in cellulose (cell) and VPDB.

In our previous study on an unexceptionally large oak dataset, it was demonstrated that there are no age trends in tree-ring stable isotopes (Büntgen et al., 2020). The raw $\delta^{13}C_{cell}$ data, obtained for individual trees with absolute dating and annual resolution, were directly used to calculate water use efficiency without the need for any additional standardization and/or correction.

2.4. *Intrinsic and evaporative water use efficiency*

To determine annual values of intrinsic water use efficiency (iWUE) based on $\delta^{13}C_{cell}$ data, the standardized approach described in Mathias and Hudiburg (2022) was followed. Firstly, we calculate the carbon isotope discrimination ($\Delta^{13}C$ in ‰) as:

$$\Delta^{13}C = \frac{\delta^{13}C_{atm} - (\delta^{13}C_{cell} - d)}{1 + \frac{(\delta^{13}C_{cell} - d)}{1000}}, \tag{2}$$

where $\delta^{13}C_{atm}$ and $\delta^{13}C_{cell}$ are the stable carbon isotope ratios in the atmosphere and tree-ring cellulose of a given year, respectively, and *d* represents correction for the post-photosynthetic fractionations (2.2 ‰). Values of $\delta^{13}C_{atm}$ reported by McCarroll and Loader (2004) and values from direct measurements on Mauna Loa (https://www.esrl.noaa.gov/gmd/dv/data/), which are implicitly assumed to be global (Belmecheri and Lavergne, 2020). Consequently, an intercellular CO_2 concentration (*C*i in μmol CO_2 mol^{-1}) was calculated as:

$$Ci = \frac{\Delta^{13}C - a - f \times \left(\frac{\Gamma^*}{pCa}\right)}{b - a} \times Ca, \tag{3}$$

where *a*, *b*, and *f* are correction factors of carbon isotope fractionations due to CO_2 diffusion across the stomata (4.4 ‰), carboxylation by Rubisco (28 ‰), and photorespiration (12 ‰), respectively, *C*a is mean annual atmospheric CO_2 concentration (μmol CO_2 mol^{-1}), while p*C*a is the equivalent of *C*a expressed as the partial pressure in Pa, and Γ* is CO_2 compensation point. Because carbon fractionation through photorespiration is temperature dependent, Γ* values were adjusted to mean temperatures of a given vegetation period and altitude (Fig. 1A, Supplementary Fig. S2A, and Supplementary Table S1) following the equations described in Bernacchi et al. (2001).

Finally, iWUE in μmol mol^{-1} was calculated according to Farquhar et al. (1989b) as:

$$iWUE = \frac{A}{g_s} = \frac{(Ca - Ci)}{1.6} \tag{4}$$

where the coefficient of 1.6 represents the ratio of stomatal conductance to CO_2 and water vapor diffusions. Values of iWUE thus represent the ratio between photosynthetic CO_2 assimilation rate (*A*) and stomatal conductance for water vapor (g_s).

In contrast, the calculation of evaporative water use efficiency (eWUE) was designed to reflect the ratio between the rates of photosynthesis and transpiration (Farquhar et al., 1989b):

$$eWUE = \frac{A}{E} = \frac{iWUE}{VD} = \frac{(Ca - Ci)}{1.6 \times VD}, \tag{5}$$

where VD is the water vapor density in the atmosphere in $kg\,m^{-3}$ converted from VPD (vapor pressure deficit) in kPa according to Strange et al. (2023) and studies cited there. Such conversion allows an accurate representation of photosynthesis (*A*) to transpiration (*E*) ratio and an expression of eWUE in mmol CO_2 mol^{-1} H_2O.

2.5. Statistical analysis

Linear regression, based on the least squares method, was used to assess changes in iWUE and eWUE over the period 1961–2020. Consequently, statistically significant differences between regression slopes for individual altitudes were evaluated using the *t*-test. Student's t-test was also employed, after testing for normality (Shapiro-Wilk test) and equality of variance (Brown-Forsythe test), to compare the 30-year periods 1961–1990 and 1991–2020 for the investigated ecophysiological and climatic parameters. SigmaPlot ver.15 software (Systat Software, San Jose, CA, US) was used for all statistical tests and graph preparation.

3. Results

All sampling sites (Table 1) are characterized by significantly different microclimatic conditions with a typical distribution of temperature, VPD and precipitations along the altitudinal gradient (Fig. 1, Supplementary Fig. S2, Supplementary Table S1). Moreover, the mean air temperature during the vegetation season significantly ($p < 0.001$) increased by 4.3–11.1 % (i.e. between 0.72 °C at the lowest altitude and 1.42 °C at the highest altitude) in the period 1991–2020 when compared with 1961–1990. Similarly, VPD values in the period 1991–2020 increased by 10.8 % at the lowest altitudes while it was by 22.0 % at high altitudes when compared to 1961–1990. Conversely, no changes in total precipitation during the growing season between these two periods were observed ($p = 0.390$–0.917). Such variability in growth conditions led to the changes in $\delta^{13}C_{cell}$ values of the tree-ring cellulose and $\delta^{13}C$-derived ecophysiological parameters (Figs. 2 and 3).

Inter-site correlations of the $\delta^{13}C_{cell}$ series revealed significant ($p < 0.05$) coherence across individual trees at the same altitude (*R*bar ranged between 0.32 and 0.38), but it was only 0.17 for *Q. petraea* trees at an altitude of 280 m a.s.l. ($p > 0.05$) over the period 1961–2020. Gradual decreases in raw $\delta^{13}C_{cell}$ values were observed during the investigated period in *Q. petraea* at all altitudes (280–630 m a.s.l.; Fig. 2A–C) and are consistent with $\delta^{13}C_{cell}$ decline in *Q. robur* (130 m a.s.l.; Supplementary Fig. S3). Such declines, being more pronounced at high than low altitudes, reflect not only the physiological adjustments of trees, but also the changes in the isotopic composition of atmospheric CO_2 ($\delta^{13}C_{atm}$) during the last decades (black lines in Fig. 2A–C).

The *Ci* time series (calculated according to Eqs. (2) and (3)) have an increasing tendency at all altitudes (Fig. 2D–F). These increases in *Ci* correspond to an increase in the atmospheric CO_2 concentration (*Ca*) from 317.4 to 412.4 µmol CO_2 mol^{-1} over the past 60 years (black line in Fig. 2D–F). While the mean *Ci* values ranged between 196 and 205 µmol CO_2 mol^{-1} over the period 1961–1990 at all altitudes (statistically non-significant differences; $p > 0.05$), *Ci* significantly increased ($p < 0.01$) in *Q. petraea* by 16.3, 14.2, and 24.1 % at the LA, MA, and HA sites, respectively, over the period 1991–2020 (Supplementary Fig. S4). However, these increases in *Ci* were not followed by a change in the *Ci*/*Ca* ratio, which remained constant (0.59–0.61) at the LA and MA sites. Noticeably, similar changes in the *Ci* and *Ci*/*Ca* ratio were observed in *Q. robur* (Supplementary Fig. S3) when compared to *Q. petraea* at the LA site; however, the absolute values for *Q. robur* are slightly higher (Supplementary Fig. S4). On the contrary, a significant ($p < 0.01$) increase in the *Ci*/*Ca* ratio by 9.8 % was found in the HA trees when comparing the

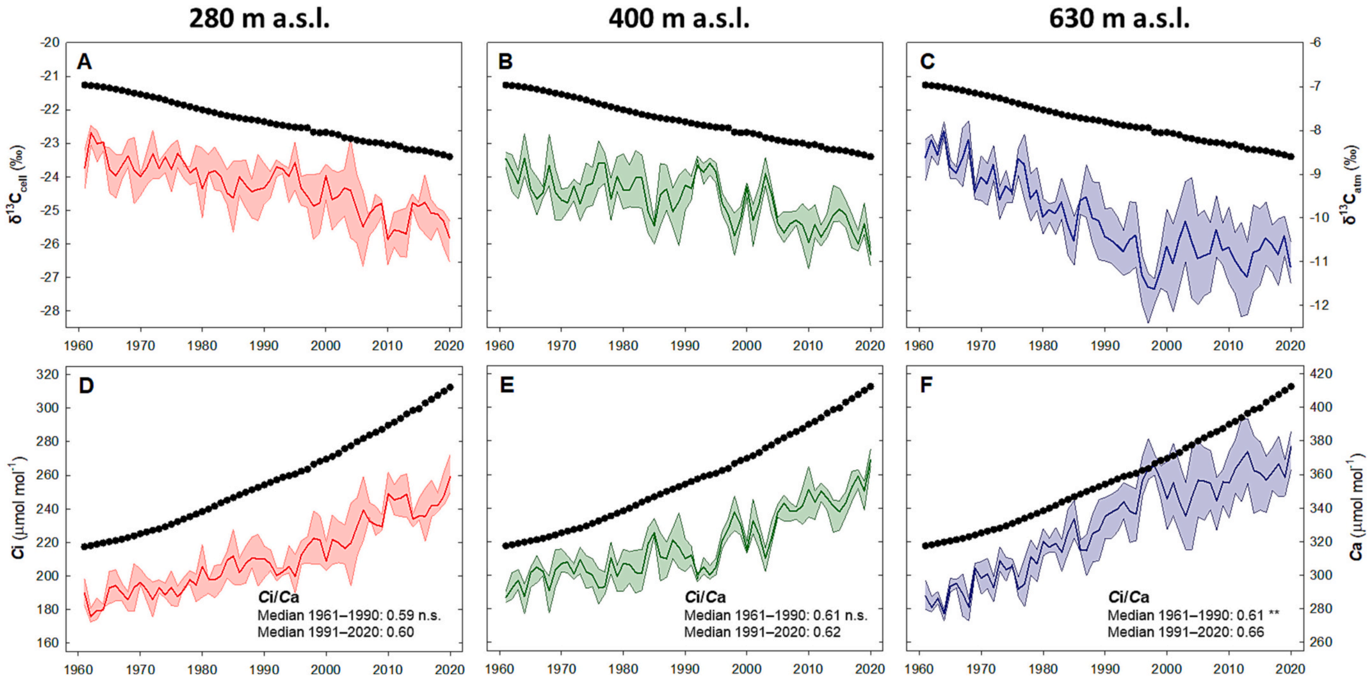

Fig. 2. Changes in stable carbon isotope ratio ($\delta^{13}C_{cell}$; A–C) and intercellular CO_2 concentration (*Ci*; D–F) over the past 60 years (1961–2020) derived from the annually resolved tree-ring cellulose of sessile oak (*Q. petraea*) trees growing at low (LA; 280 m a.s.l.), middle (MA; 400 m a.s.l.), and high altitude (HA; 630 m a.s.l.). Coloured lines represent the mean values ($n = 6$), while coloured areas indicate the range between the first and the third quartiles. Black lines show (A–C) stable carbon isotope ratio in the atmosphere ($\delta^{13}C_{atm}$) and (D–F) mean annual atmospheric CO_2 concentration (*Ca*). Statistical differences in *Ci*/*Ca* ratio between the periods 1961–1990 and 1991–2020 are shown in figs. D–F: n.s. = statistically non-significant difference ($p > 0.05$), ** = statistically highly significant difference ($p < 0.01$).

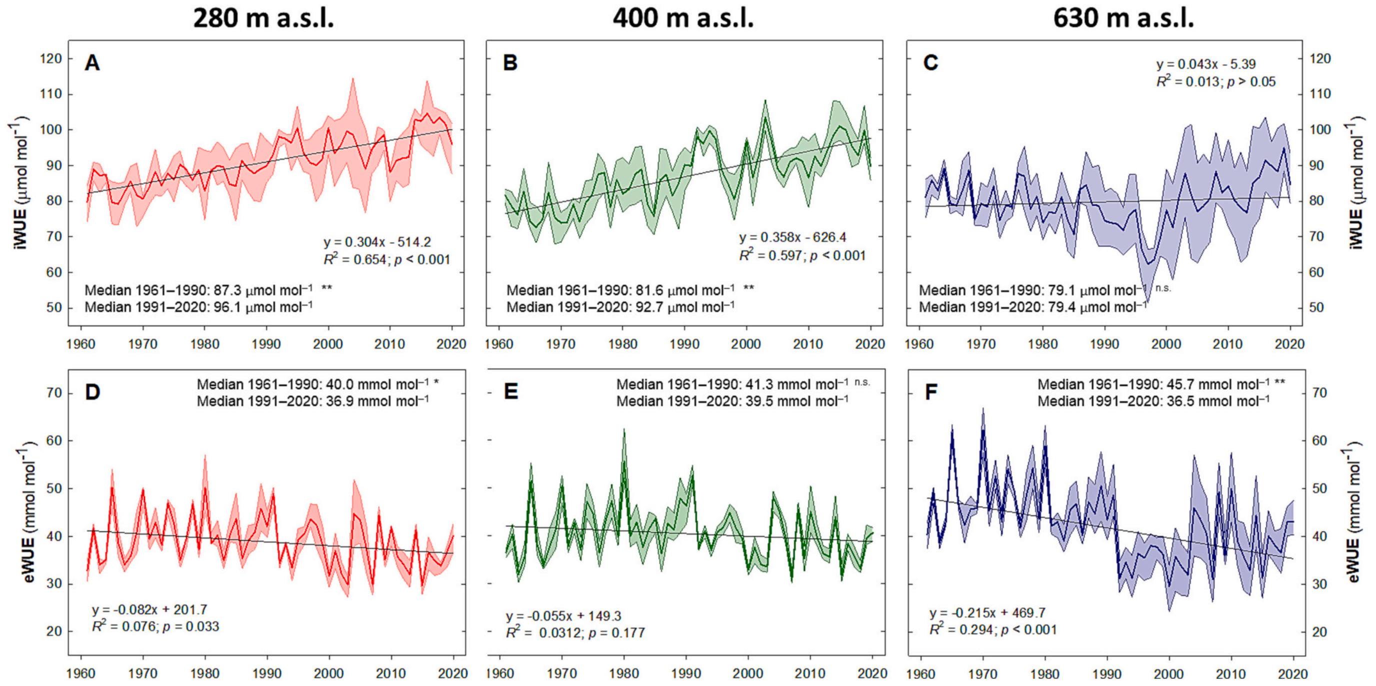

Fig. 3. Changes in intrinsic water use efficiency (iWUE; A–C) and evaporative water use efficiency (eWUE; D–F) over the past 60 years (1961–2020) derived from the annually resolved tree-ring cellulose of sessile oak (*Q. petraea*) trees growing at low (LA; 280 m a.s.l.), middle (MA; 400 m a.s.l.), and high altitude (HA; 630 m a.s.l.). Coloured lines represent the mean values ($n = 6$), while coloured areas indicate the range between the first and the third quartiles. Statistical differences in iWUE (A–C) and eWUE (D–F) values between the periods 1961–1990 and 1991–2020 are shown: n.s. = statistically non-significant difference ($p > 0.05$), * = statistically significant difference ($p < 0.05$), ** = statistically highly significant difference ($p < 0.01$).

1961–1990 (mean *Ci*/*Ca* value 0.61) and 1991–2020 periods (mean *Ci*/*Ca* value 0.67). Generally, the highest *Ci*/*Ca* values (0.726) were found in cold years (1997), while the lowest *Ci*/*Ca* values (0.553) were observed in dry years (1964).

During the investigated period, iWUE linearly increased in *Q. robur* (Supplementary Fig. S3) as well as *Q. petraea* trees from the LA and MA sites, but not in those from the HA (Fig. 3A–C). The slope of linear regression is significantly ($p < 0.01$) lower at the HA site (increase by 0.43 µmol mol^{-1} per decade) when compared to LA and MA ones (increase by 3.04–3.58 µmol mol^{-1} per decade). Noticeably, the highest increase in iWUE (4.52 µmol mol^{-1} per decade) was found in *Q. robur* trees at the altitude of 130 m a.s.l. (Supplementary Fig. S3). At low altitude (Fig. 3A), the iWUE values of *Q. petraea* varied between 79.2 µmol mol^{-1} in 1966 (wet year) and 104.7 µmol mol^{-1} in 2016 (hot and dry year; mean seasonal temperature > 15.5 °C and total seasonal precipitation <345 mm). The same pattern was observed for *Q. robur*. Also at middle and high altitudes, the minimal iWUE values (72.7 and 62.3 µmol mol^{-1} for MA and HA sites, respectively) were associated with wet (total seasonal precipitation >475 mm) and/or cold years (mean seasonal temperature < 13.8 °C), while maximum iWUE values (103.6 and 94.9 µmol mol^{-1} for MA and HA sites, respectively) have been achieved in hot and dry years (Fig. 3B, C; Supplementary Table S2). In line with these results, a comparison of mean iWUE values over the 1961–1990 and 1991–2020 periods revealed significant ($p < 0.01$) increases by 17.3 % in *Q. robur* and by 11.8 and 14.9 % in *Q. petraea* trees grown at the LA and MA sites, respectively, but no change in the HA trees was observed (Supplementary Fig. S5).

The mean eWUE values for the investigated period were 38.9 mmol mol^{-1} for LA trees, 40.5 for MA trees, and 42.6 for HA trees (*Q. petraea*). However, the lowest eWUE value of 32.3 mmol mol^{-1} was observed for *Q. robur* grown at the lowest altitude. These differences among sites are statistically significant ($p < 0.01$). Compared to iWUE, different trends in eWUE development during the period 1961–2020 were observed (Fig. 3D–F). Although weak or no trends were observed in the LA and MA trees, a significant ($p < 0.01$; $r = 0.499$) decrease in eWUE was observed in the HA trees. The mean eWUE value amounted 47.1 mmol mol^{-1} during the 1961–1990 period while it was only 38.1 mmol mol^{-1} during 1991–2020, i.e. a significant ($p < 0.01$) decrease by 19.1 % (Supplementary Fig. S5).

At all altitudes, the minimal eWUE values (29.7, 31.1, and 29.6 mmol mol^{-1} at LA, MA, and HA, respectively) were associated with hot and dry years, while maximum values (50.4, 55.7, and 62.39 mmol mol^{-1} at LA, MA, and HA, respectively) were found in cold and wet years.

4. Discussion

4.1. Changes in climate conditions along the altitudinal gradient

In Europe, ongoing climate change, besides an increase in average temperature, is often marked by prolonged periods of drought and heatwaves (Brázdil et al., 2021; Seneviratne et al., 2021). However, these trends vary with altitude (Kotlarski et al., 2012, 2015). Generally, warming intensifies with altitude, while the influence of altitude on precipitation changes is relatively minor. These patterns align with the observed trends in air temperature and precipitation during the vegetation period (April–August) in our study area (Fig. 1 and Supplementary Fig. S2). Considering the anticipated ongoing rise in temperature and VPD in Central Europe (Treydte et al., 2024), the observed reactions of lowland trees to current climate conditions can serve as a valuable indicator of future behavior and the long-term acclimation capacity of highland trees (Leroy et al., 2020). Additionally, it can be utilized as a predictor for potential alterations in highland forest biodiversity, structure, or treeline shifts (Körner, 2007). Notably, a more significant increase in atmospheric VPD occurred at higher altitudes (an increase in atmospheric VPD by 10.8 % (130 m a.s.l.) and 22.0 % (630 m a.s.l.) comparing the periods 1991–2020 to those of 1960–1990). This suggests a potentially stronger impact of climate change on eWUE at higher altitudes.

4.2. *Environmental changes improve iWUE but not eWUE values*

The range of observed $\delta^{13}C_{cell}$ values in our study (Fig. 2A–C) is consistent with values reported for oak species at different European sites (ISONET Project Members et al., 2023). The isotope-ratio mass spectrometry was shown to be sensitive enough to distinguish between trees growing along a relatively small altitudinal gradient. Our 60-year (1961–2020) dataset based on $\delta^{13}C_{cell}$ values supports the hypothesis that ongoing environmental changes (associated with increasing CO_2 concentration, temperature, and drought) lead to increased water use efficiency in oak trees. This effect is particularly noticeable for oak trees at low and middle altitudes, specifically concerning intrinsic iWUE, reflecting changes in the stomatal aperture openness (Fig. 3A, B). In addition to climatic factors, it is likely that the natural hybridization of *Q. robur* and *Q. petraea* contributes to a gradual improvement of water status and greater resistance of oaks to drought (Petit et al., 2003).

Similar findings documenting an increase in iWUE during recent decades have been reported for various tree species including oaks, pine, beech, and spruce (Dorado-Liñán et al., 2020; Kannenberg et al., 2021; Peñuelas et al., 2008; Waterhouse et al., 2004; Wu et al., 2015). For example, Dorado-Liñán et al. (2020) reported an increase of mean iWUE values from 64.8 (period 1880–1915) to 81.1 μmol mol^{-1} (period 1980–2015) in high-altitude (1500 m a.s.l.) *Q. petraea* tress. Such iWUE values are comparable to those reported in this study for HA trees in the period 1961–2020 (62.3–95.0 μmol mol^{-1}; Fig. 3C). Similarly, the iWUE values observed for LA trees (71–127 μmol mol^{-1} for *Q. robur* and 72–122 μmol mol^{-1} for *Q. petraea*) are in line with those previously presented for *Q. robur* growing at altitudes of 50–150 m a.s.l. (75–115 μmol mol^{-1}; Waterhouse et al., 2004). A cross-European study by Frank et al. (2015) also noted that iWUE values increase more rapidly in coniferous than broadleaved tree species, with the highest increases in temperate forests of Central Europe. However, a decline in iWUE has been noted in Silver fir during the last 30 years, which was probably related to the deposition of sulfur and nitrogen in Central Europe and consequent changes in the availability of micronutrients including calcium (Oulehle et al., 2023). Similarly, Bauters et al. (2020) reported a decline in iWUE for tropical trees in central Africa over the past century, likely due to significant nutritional constraints on forest growth and productivity.

While $\delta^{13}C_{cell}$ values in oak tree rings are generally considered independent of cambial age (Büntgen et al., 2020), an increase in iWUE may occur with tree age, especially when comparing small juvenile trees with tall middle-aged and mature trees (Wu et al., 2018). Such an increase is often attributed to rising hydraulic stress associated with increasing tree size, leading to reduced stomatal conductance (reviewed in Bond, 2000). However, it is anticipated that the age trends of iWUE in our study will be relatively low because the average tree age at the beginning of the study (1961) was >60 years (Table 1). The meta-analysis by Mathias and Thomas (2021) revealed that the increase in iWUE is primarily modulated by increasing the CO_2 assimilation rate (observed in 83 % of the examined studies), while the reduced stomatal conductance played the dominant role in only 17 % of the examined studies. The stimulatory effect is mainly attributed to elevated CO_2 concentration in the atmosphere leading to an increase in the CO_2 assimilation rate and a reduction of stomatal conductance (Poorter et al., 2022; Urban, 2003; Weiwei et al., 2018). However, the stimulating effect of CO_2 on photosynthesis can be negligible in cold, dry, and/or nutrient-poor environments (Albert et al., 2011; Delgado et al., 1994; Hättenschwiler and Körner, 1997; Ofori-Amanfo et al., 2023; Poorter et al., 2022; Šigut et al., 2015). Unlike iWUE, the eWUE time series (Fig. 3D–F) did not show any increasing trends in evaporation-based water use efficiency over the last 60 years. This result indicates that the positive effects of elevated CO_2 concentration and temperature on photosynthesis and stomatal closure, leading to increased iWUE, might be suppressed by the increasing deficit of water vapor in the atmosphere (Fig. 1). This deficit accelerates transpiration rates through plants, potentially resulting in higher transpiration even when stomata are partially or fully closed (Marchin et al., 2016; Leonardi et al., 2000).

The different trends between iWUE and eWUE suggest the necessity of considering changes in atmospheric VPD in WUE calculations, especially at ecosystem and global scales. Furthermore, it emphasizes the importance of accurate interpretation of the WUE based on stomatal conductance or transpiration rate. iWUE appears to be a robust indicator of long-term ecophysiological responses to climate change and CO_2 concentration, while eWUE is closely linked with environmental impacts on plant water use and directly related to water and carbon cycles (Strange et al., 2023).

4.3. *Trees acclimate to environmental changes differently at different altitudes*

Our data support the hypothesis that changes in WUE over the past 60 years vary along the altitudinal gradient. We observed a significant increase in iWUE in low-altitude oak trees (by 26.9 % in *Q. robur* and by 20.3 % in *Q. petraea*), whereas this increase was marginal in high-altitude *Q. petraea* trees (by 4.4 %; Fig. 3A, C and Supplementary Fig. S3). Similar trends in iWUE time series were observed for European beech in the Montseny Mountains, Spain (Peñuelas et al., 2008), and Schrenk spruce in the Tienshan Mountains, China (Wu et al., 2015). For the period 1978–2003, Peñuelas et al. (2008) reported an increase in $\delta^{13}C$-based iWUE values by 10 % and 6 % at altitudes of 990 and 1130 m a.s.l., respectively, while no increasing trend in iWUE was observed at high altitudes (1640 m a.s.l.). The more significant increase in iWUE at lower altitudes is primarily attributed to warmer and drier conditions, which regulate stomatal aperture and maintain high photosynthetic CO_2 uptake efficiency stimulated by increasing CO_2 concentration (Rajsnerová et al., 2015; Zhang et al., 2005). On the contrary, the stimulatory effect of elevated CO_2 on photosynthesis is minimized at suboptimally low temperatures typical for high altitudes (Bernacchi et al., 2001; Šigut et al., 2015; Zhang et al., 2005), thus contributing to a smaller increase in iWUE. This reduction in photosynthetic efficiency may lead to a progressive rise in intercellular CO_2 concentration (Farquhar et al., 1982; Zhang et al., 2005), supported by a significant increase in *Ci*/*Ca* ratio at high altitudes over the past 30 years (average increase of 9.8 %). On the contrary, the ratio remained relatively stable at low and middle altitudes (average increase of up to 1.7 %) compared to the 1961–1990 period (Supplementary Fig. S4). Similar trends in *Ci*/*Ca* ratio along the altitudinal gradient were reported for both broadleaved (Peñuelas et al., 2008) and coniferous tree species (Wu et al., 2015).

However, contrasting trends were observed in eWUE (Fig. 3), especially in high-altitude trees, where a nearly 20 % decrease in eWUE corresponded to a 22 % increase in atmospheric VPD (Fig. 1). This finding implies a lower ability of trees to cope with changing conditions, higher water loss at the ecosystem level due to stimulated transpiration, and thus a growing risk of drought stress for forest stands at higher altitudes, a risk previously considered low. Opposing trends between iWUE and eWUE were also observed in forest populations during the megadrought in the southwest of the US (Strange et al., 2023). Trees accessing underground water levels operated with lower iWUE and higher eWUE values, indicating less physiological stress compared to trees at dry sites.

To our knowledge, this is the first study demonstrating the development of eWUE along an altitudinal gradient based on $\delta^{13}C_{cell}$ values in tree rings. Several studies combining CO_2 and H_2O flux measurements with modeling approaches have shown an increase in WUE at the ecosystem level with altitude. However, at high altitudes, where adverse conditions for growth, photosynthesis, and transpiration prevail, ecosystem WUE may decrease, as reported for forest (Sun et al., 2020) and grassland ecosystems (Han et al., 2013). Parametrization of a coupled model for evapotranspiration and gross primary production using satellite MODIS products revealed a slight increase in WUE up to altitudes of 1100 m a.s.l. in China, irrespective of vegetation cover type (Nandy et al., 2022; Zhang et al., 2023). However, WUE dramatically decreased at altitudes above 5000 m a.s.l. (Xue et al., 2015).

5. Conclusions

In this study, we have confirmed significant differences in climate factors along the natural altitudinal gradient (130–630 m a.s.l.) where oak species grow in Central Europe. Amplified atmosphere warming and desiccation increase significantly with altitude, particularly in the last three decades. Changes in total precipitation during the vegetation season remain relatively stable. These climatic shifts, combined with increasing CO_2 concentration, have influenced intrinsic (stomata-based) and evaporative (transpiration-based) WUE, determined through a highly sensitive detection of stable carbon isotope ratios in the latewood cellulose ($\delta^{13}C_{cell}$) of annually resolved tree rings. While iWUE significantly increased in the last 60 years in oaks grown at low (irrespective of *Quercus* species) and middle altitudes, this trend was negligible at high altitudes. The marked increase in iWUE at lower altitudes suggests more efficient photosynthesis and stomatal closure in response to warmer and drier conditions. On the contrary, the stimulatory effects of elevated CO_2 and temperature on iWUE appear to be limited by the rising deficit of water vapor in the atmosphere at higher altitudes.

In contrast to iWUE, eWUE did not exhibit any increasing trends over the past 60 years. A slight decrease in eWUE was observed in high-altitude trees, corresponding to a 22 % increase in atmospheric VPD. These divergent trends between iWUE and eWUE emphasize the importance of accurate interpretation of these two measures of plant physiological status. Furthermore, they underscore the necessity of correcting for atmospheric VPD changes, especially when evaluating changes in WUE on ecosystem and global scales.

CRediT authorship contribution statement

Natálie Pernicová: Writing – original draft, Methodology, Formal analysis, Conceptualization. **Otmar Urban:** Writing – original draft, Visualization, Investigation, Formal analysis, Conceptualization. **Josef Čáslavský:** Validation, Methodology, Formal analysis. **Tomáš Kolář:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Michal Rybníček:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Irena Sochová:** Writing – review & editing, Methodology, Investigation. **Josep Peñuelas:** Writing – review & editing, Writing – original draft, Supervision, Investigation, Conceptualization. **Michal Bošeľa:** Data curation, Methodology, Writing – review & editing. **Miroslav Trnka:** Writing – review & editing, Validation, Supervision, Investigation, Data curation.

Declaration of competing interest

All authors declare no competing interests.

Data availability

Primary datasets of climate characteristics and carbon isotopic ratios are openly available in a public repository ASEP (operated by the Czech Academy of Sciences): https://doi.org/10.57680/asep.0583155.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.171173.

References

Adams, M.A., Buckley, T.N., Turnbull, T.L., 2020. Diminishing CO_2-driven gains in water-use efficiency of global forests. Nat. Clim. Chang. 10 (5), 466–471. https://doi.org/10.1038/s41558-020-0747-7.

Albert, K.R., Ro-Poulsen, H., Mikkelsen, T.N., Michelsen, A., Van Der Linden, L., Beier, C., 2011. Effects of elevated CO_2, warming and drought episodes on plant carbon uptake in a temperate heath ecosystem are controlled by soil water status. Plant Cell Environ. 34 (7), 1207–1222. https://doi.org/10.1111/j.1365-3040.2011.02320.x.

Allen, R.G., Pereira, L.S., Raes, D., Smith, M., 1998. Crop Evapotranspiration. Guidelines for Computing Crop Water Requirements. FAO Irrigation and Drainage Paper 56. Rome, Italy, FAO–Food and Agriculture Organization of the United Nations, p. 290.

Andreu-Hayles, L.A.I.A., Planells, O., Gutierrez, E., Muntan, E., Helle, G., Anchukaitis, K. J., Schleser, G.H., 2011. Long tree-ring chronologies reveal 20th century increases in water-use efficiency but no enhancement of tree growth at five Iberian pine forests. Glob. Chang. Biol. 17 (6), 2095–2112. https://doi.org/10.1111/j.1365-2486.2010.02373.x.

Bauters, M., Meeus, S., Barthel, M., Stoffelen, P., De Deurwaerder, H.P., Meunier, F., Boeckx, P., 2020. Century-long apparent decrease in intrinsic water-use efficiency with no evidence of progressive nutrient limitation in African tropical forests. Glob. Chang. Biol. 26 (8), 4449–4461. https://doi.org/10.1111/gcb.15145.

Belmecheri, S., Lavergne, A., 2020. Compiled records of atmospheric CO_2 concentrations and stable carbon isotopes to reconstruct climate and derive plant ecophysiological indices from tree rings. Dendrochronologia 63, 125748. https://doi.org/10.1016/j.dendro.2020.125748.

Bernacchi, C.J., Singsaas, E.L., Pimentel, C., Portis Jr., A.R., Long, S.P., 2001. Improved temperature response functions for models of Rubisco-limited photosynthesis. Plant Cell Environ. 24 (2), 253–259. https://doi.org/10.1111/j.1365-3040.2001.00668.x.

Boettger, T., Haupt, M., Knöller, K., Weise, S.M., Waterhouse, J.S., Rinne, K.T., Schleser, G.H., 2007. Wood cellulose preparation methods and mass spectrometric analyses of $\delta^{13}C$, $\delta^{18}O$, and nonexchangeable δ^2H values in cellulose, sugar, and starch: an interlaboratory comparison. Anal. Chem. 79 (12), 4603–4612. https://doi.org/10.1021/ac0700023.

Bond, B.J., 2000. Age-related changes in photosynthesis of woody plants. Trends Plant Sci. 5 (8), 349–353. https://doi.org/10.1016/S1360-1385(00)01691-5.

Bošeľa, M., Sedmák, R., Sedmáková, D., Marušák, R., Kulla, L., 2014. Temporal shifts of climate–growth relationships of Norway spruce as an indicator of health decline in the Beskids, Slovakia. For. Ecol. Manag. 325, 108–117. https://doi.org/10.1016/j.foreco.2014.03.055.

Bosel'a, M., Tumajer, J., Cienciala, E., Dobor, L., Kulla, L., Marčiš, P., Büntgen, U., 2021. Climate warming induced synchronous growth decline in Norway spruce populations across biogeographical gradients since 2000. Sci. Total Environ. 752, 141794 https://doi.org/10.1016/j.scitotenv.2020.141794.

Brázdil, R., Zahradníček, P., Dobrovolný, P., Štěpánek, P., Trnka, M., 2021. Observed changes in precipitation during recent warming: the Czech Republic, 1961–2019. Int. J. Climatol. 41 (7), 3881–3902. https://doi.org/10.1002/joc.7048.

Bresson, C.C., Kowalski, A.S., Kremer, A., Delzon, S., 2009. Evidence of altitudinal increase in photosynthetic capacity: gas exchange measurements at ambient and constant CO_2 partial pressures. Ann. For. Sci. 66 (5), 1–8. https://doi.org/10.1051/forest/2009027.

Bresson, C.C., Vitasse, Y., Kremer, A., Delzon, S., 2011. To what extent is altitudinal variation of functional traits driven by genetic adaptation in European oak and beech? Tree Physiol. 31 (11), 1164–1174. https://doi.org/10.1093/treephys/tpr084.

Büntgen, U., Kolář, T., Rybníček, M., Koňasová, E., Trnka, M., Ač, A., Krusic, P.J., Esper, J., Treydte, K., Reinig, F., Kirdyanov, A., Herzig, F., Urban, O., 2020. No age trends in oak stable isotopes. Paleoceanogr. Paleoclimatol. 35 (4) https://doi.org/10.1029/2019PA003831.

Cheng, L., Zhang, L., Wang, Y.P., Canadell, J.G., Chiew, F.H., Beringer, J., Zhang, Y., 2017. Recent increases in terrestrial carbon uptake at little cost to the water cycle. Nat. Commun. 8 (1), 110. https://doi.org/10.1038/s41467-017-00114-5.

De Frenne, P., Graae, B.J., Rodríguez-Sánchez, F., Kolb, A., Chabrerie, O., Decocq, G., Verheyen, K., 2013. Latitudinal gradients as natural laboratories to infer species' responses to temperature. J. Ecol. 101 (3), 784–795. https://doi.org/10.1111/1365-2745.12074.

Delgado, E., Mitchell, R.A.C., Parry, M.A.J., Driscoll, S.P., Mitchell, V.J., Lawlor, D.W., 1994. Interacting effects of CO_2 concentration, temperature and nitrogen supply on the photosynthesis and composition of winter wheat leaves. Plant Cell Environ. 17 (11), 1205–1213. https://doi.org/10.1111/j.1365-3040.1994.tb02018.x.

Dijkstra, F.A., Carrillo, Y., Aspinwall, M.J., Maier, C., Canarini, A., Tahaei, H., Choat, B., Tissue, D.T., 2016. Water, nitrogen and phosphorus use efficiencies of four tree species in response to variable water and nutrient supply. Plant Soil 406, 187–199. https://doi.org/10.1007/s11104-016-2873-6.

Dorado-Liñán, I., Valbuena-Carabaña, M., Cañellas, I., Gil, L., Gea-Izquierdo, G., 2020. Climate change synchronizes growth and iWUE across species in a temperate-submediterranean mixed oak forest. Front. Plant Sci. 11, 706. https://doi.org/10.3389/fpls.2020.00706.

Eaton, E.G.S.D.J., Caudullo, G., Oliveira, S., De Rigo, D., 2016. *Quercus robur* and *Quercus petraea* in Europe: distribution, habitat, usage and threats. In: San-Miguel-Ayanz, J., de Rigo, D., Caudullo, G., Houston Durrant, T., Mauri, A. (Eds.), European Atlas of Forest Tree Species. Public Office of the European Union, Luxembourg, pp. 160–163.

Ehleringer, J.R., Hall, A.E., Farquhar, G.D., 1993. Introduction: water use in relation to productivity. In: Ehleringer, J.R., Hall, A.E., Farquhar, G.D. (Eds.), Stable Isotopes and Plant Carbon-Water Relations. Academic Press, New York, pp. 3–8.

El Masri, B., Schwalm, C., Huntzinger, D.N., Mao, J., Shi, X., Peng, C., Michalak, A.M., 2019. Carbon and water use efficiencies: a comparative analysis of ten terrestrial ecosystem models under changing climate. Sci. Rep. 9 (1), 14680 https://doi.org/10.1038/s41598-019-50808-7.

Farquhar, G.D., O'Leary, M.H., Berry, J.A., 1982. On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. Funct. Plant Biol. 9 (2), 121–137. https://doi.org/10.1071/PP9820121.

Farquhar, G.D., Ehleringer, J.R., Hubick, K.T., 1989a. Carbon isotope discrimination and photosynthesis. Annu. Rev. Plant Biol. 40 (1), 503–537. https://doi.org/10.1146/annurev.pp.40.060189.002443.

Farquhar, G.D., Hubick, K.T., Condon, A.G., Richards, R.A., 1989b. Carbon isotope fractionation and plant water-use efficiency. In: Rundel, P.W., Ehleringer, J.R., Nagy, K.A. (Eds.), Stable Isotopes in Ecological Research. Springer, New York, NY, pp. 21–40.

Fletcher, A.L., Sinclair, T.R., Allen Jr., L.H., 2007. Transpiration responses to vapor pressure deficit in well watered 'slow-wilting' and commercial soybean. Environ. Exp. Bot. 61 (2), 145–151. https://doi.org/10.1016/j.envexpbot.2007.05.004.

Frank, D.C., Poulter, B., Saurer, M., Esper, J., Huntingford, C., Helle, G., Weigl, M., 2015. Water-use efficiency and transpiration across European forests during the Anthropocene. Nat. Clim. Chang. 5 (6), 579–583. https://doi.org/10.1038/nclimate2614.

Giammarchi, F., Cherubini, P., Pretzsch, H., Tonon, G., 2017. The increase of atmospheric CO_2 affects growth potential and intrinsic water-use efficiency of Norway spruce forests: insights from a multi-stable isotope analysis in tree rings of two Alpine chronosequences. Trees 31, 503–515. https://doi.org/10.1007/s00468-016-1478-2.

Grossiord, C., Buckley, T.N., Cernusak, L.A., Novick, K.A., Poulter, B., Siegwolf, R.T., McDowell, N.G., 2020. Plant responses to rising vapor pressure deficit. New Phytol. 226 (6), 1550–1566. https://doi.org/10.1111/nph.16485.

Guerrieri, R., Belmecheri, S., Ollinger, S.V., Asbjornsen, H., Jennings, K., Xiao, J., Richardson, A.D., 2019. Disentangling the role of photosynthesis and stomatal conductance on rising forest water-use efficiency. Proc. Natl. Acad. Sci. 116 (34), 16909–16914. https://doi.org/10.1073/pnas.1905912116.

Gut, U., Árvai, M., Bijak, S., Camarero, J.J., Cedro, A., Cruz-García, R., Buras, A., 2019. No systematic effects of sampling direction on climate-growth relationships in a large-scale, multi-species tree-ring data set. Dendrochronologia 57, 125624. https://doi.org/10.1016/j.dendro.2019.125624.

Han, Q., Luo, G., Li, C., Ye, H., Chen, Y., 2013. Modeling grassland net primary productivity and water-use efficiency along an elevational gradient of the Northern Tianshan Mountains. J. Arid. Land 5, 354–365. https://doi.org/10.1007/s40333-013-0182-y.

Hartl, C., Schneider, L., Riechelmann, D.F., Kuhl, E., Kochbeck, M., Klippel, L., Esper, J., 2022. The temperature sensitivity along elevational gradients is more stable in maximum latewood density than tree-ring width. Dendrochronologia 73, 125958. https://doi.org/10.1016/j.dendro.2022.125958.

Hartl-Meier, C., Zang, C., Büntgen, U., Esper, J., Rothe, A., Göttlein, A., Treydte, K., 2015. Uniform climate sensitivity in tree-ring stable isotopes across species and sites in a mid-latitude temperate forest. Tree Physiol. 35 (1), 4–15. https://doi.org/10.1093/treephys/tpu096.

Hättenschwiler, S., Körner, C., 1997. Biomass allocation and canopy development in spruce model ecosystems under elevated CO_2 and increased N deposition. Oecologia 113, 104–114. https://doi.org/10.1007/s004420050358.

Hill, S.A., Waterhouse, J.S., Field, E.M., Switsur, V.R., Ap Rees, T., 1995. Rapid recycling of triose phosphates in oak stem tissue. Plant Cell Environ. 18 (8), 931–936. https://doi.org/10.1111/j.1365-3040.1995.tb00603.x.

ISONET Project Members, Schleser, G.H., Andreu-Hayles, L., Bednarz, Z., Berninger, F., Boettger, T., Wimmer, R., 2023. Stable carbon isotope ratios of tree-ring cellulose from the site network of the EU-Project 'ISONET'. In: GFZ Data Services https://doi.org/10.5880/GFZ.4.3.2023.002.

Jump, A.S., Mátyás, C., Peñuelas, J., 2009. The altitude-for-latitude disparity in the range retractions of woody species. Trends Ecol. Evol. 24 (12), 694–701. https://doi.org/10.1016/j.tree.2009.06.007.

Kannenberg, S.A., Driscoll, A.W., Szejner, P., Anderegg, W.R., Ehleringer, J.R., 2021. Rapid increases in shrubland and forest intrinsic water-use efficiency during an ongoing megadrought. Proc. Natl. Acad. Sci. 118 (52), e2118052118 https://doi.org/10.1073/pnas.2118052118.

King, G.M., Gugerli, F., Fonti, P., Frank, D.C., 2013. Tree growth response along an elevational gradient: climate or genetics? Oecologia 173, 1587–1600. https://doi.org/10.1007/s00442-013-2696-6.

Kolb, T.E., Stone, J.E., 2000. Differences in leaf gas exchange and water relations among species and tree sizes in an Arizona pine–oak forest. Tree Physiol. 20 (1), 1–12. https://doi.org/10.1093/treephys/20.1.1.

Körner, C., 2007. The use of 'altitude'in ecological research. Trends Ecol. Evol. 22 (11), 569–574. https://doi.org/10.1016/j.tree.2007.09.006.

Kotlarski, S., Bosshard, T., Lüthi, D., Pall, P., Schär, C., 2012. Elevation gradients of European climate change in the regional climate model COSMO-CLM. Clim. Chang. 112, 189–215. https://doi.org/10.1007/s10584-011-0195-5.

Kotlarski, S., Lüthi, D., Schär, C., 2015. The elevation dependency of 21st century European climate change: an RCM ensemble perspective. Int. J. Climatol. 35 (13), 3902–3920. https://doi.org/10.1002/joc.4254.

Kubiske, M.E., Pregitzer, K.S., 1996. Effects of elevated CO_2 and light availability on the photosynthetic light response of trees of contrasting shade tolerance. Tree Physiol. 16 (3), 351–358. https://doi.org/10.1093/treephys/16.3.351.

Leonardi, C., Guichard, S., Bertin, N., 2000. High vapour pressure deficit influences growth, transpiration and quality of tomato fruits. Sci. Hortic. 84 (3–4), 285–296. https://doi.org/10.1016/S0304-4238(99)00127-2.

Leroy, T., Louvet, J.M., Lalanne, C., Le Provost, G., Labadie, K., Aury, J.M., Kremer, A., 2020. Adaptive introgression as a driver of local adaptation to climate in European white oaks. New Phytol. 226 (4), 1171–1182. https://doi.org/10.1111/nph.16095.

Lévesque, M., Siegwolf, R., Saurer, M., Eilmann, B., Rigling, A., 2014. Increased water-use efficiency does not lead to enhanced tree growth under xeric and mesic conditions. New Phytol. 203 (1), 94–109. https://doi.org/10.1111/nph.12772.

Marchin, R.M., Broadhead, A.A., Bostic, L.E., Dunn, R.R., Hoffmann, W.A., 2016. Stomatal acclimation to vapour pressure deficit doubles transpiration of small tree seedlings with warming. Plant Cell Environ. 39 (10), 2221–2234. https://doi.org/10.1111/pce.12790.

Mathias, J.M., Hudiburg, T.W., 2022. isocalcR: an R package to streamline and 587 standardize stable isotope calculations in ecological research. Glob. Chang. Biol. 28 (24), 7428–7436. https://doi.org/10.1111/gcb.16407.

Mathias, J.M., Thomas, R.B., 2021. Global tree intrinsic water use efficiency is enhanced by increased atmospheric CO_2 and modulated by climate and plant functional types. Proc. Natl. Acad. Sci. 118 (7), e2014286118 https://doi.org/10.1073/pnas.2014286118.

McCarroll, D., Loader, N.J., 2004. Stable isotopes in tree rings. Quat. Sci. Rev. 23 (7–8), 771–801. https://doi.org/10.1016/j.quascirev.2003.06.017.

McDowell, N.G., White, S., Pockman, W.T., 2008. Transpiration and stomatal conductance across a steep climate gradient in the southern Rocky Mountains. Ecohydrology 1 (3), 193–204. https://doi.org/10.1002/eco.20.

Mediavilla, S., González-Zurdo, P., Babiano, J., Escudero, A., 2016. Responses of photosynthetic parameters to differences in winter temperatures throughout a temperature gradient in two evergreen tree species. Eur. J. For. Res. 135, 871–883. https://doi.org/10.1007/s10342-016-0980-9.

Moore, C.E., Meacham-Hensold, K., Lemonnier, P., Slattery, R.A., Benjamin, C., Bernacchi, C.J., Cavanagh, A.P., 2021. The effect of increasing temperature on crop photosynthesis: from enzymes to ecosystems. J. Exp. Bot. 72 (8), 2822–2844. https://doi.org/10.1093/jxb/erab090.

Nandy, S., Saranya, M., Srinet, R., 2022. Spatio-temporal variability of water use efficiency and its drivers in major forest formations in India. Remote Sens. Environ. 269, 112791 https://doi.org/10.1016/j.rse.2021.112791.

Ofori-Amanfo, K.K., Klem, K., Veselá, B., Holub, P., Agyei, T., Juráň, S., Urban, O., 2023. The effect of elevated CO_2 on photosynthesis is modulated by nitrogen supply and reduced water availability in *Picea abies*. Tree Physiol. 43 (6), 925–937. https://doi.org/10.1093/treephys/tpad024.

Osmond, C.B., Björkman, O., Anderson, D.J., 1980. Photosynthesis. In: Osmond, C.B., Björkman, O., Anderson, D.J. (Eds.), Physiological Processes in Plant Ecology. Toward a Synthesis with Atriplex. Springer Berlin, Heidelberg, pp. 291–377. https://doi.org/10.1007/978-3-642-67637-6.

Oulehle, F., Urban, O., Tahovská, K., Kolář, T., Rybníček, M., Büntgen, U., Trnka, M., 2023. Calcium availability affects the intrinsic water-use efficiency of temperate forest trees. Commun. Earth Environ. 4 (1), 199. https://doi.org/10.1038/s43247-023-00822-5.

Pan, Y., Birdsey, R.A., Fang, J., Houghton, R., Kauppi, P.E., Kurz, W.A., Hayes, D., 2011. A large and persistent carbon sink in the world's forests. Science 333 (6045), 988–993. https://doi.org/10.1126/science.120160.

Paulsen, J., Weber, U.M., Körner, C., 2000. Tree growth near treeline: abrupt or gradual reduction with altitude? Arct. Antarct. Alp. Res. 32 (1), 14–20. https://doi.org/10.1080/15230430.2000.12003334.

Peñuelas, J., Hunt, J.M., Ogaya, R., Jump, A.S., 2008. Twentieth century changes of tree-ring $\delta^{13}C$ at the southern range-edge of *Fagus sylvatica*: increasing water-use efficiency does not avoid the growth decline induced by warming at low altitudes. Glob. Chang. Biol. 14 (5), 1076–1088. https://doi.org/10.1111/j.1365-2486.2008.01563.x.

Petit, R.J., Bodénès, C., Ducousso, A., Roussel, G., Kremer, A., 2003. Hybridization as a mechanism of invasion in oaks. New Phytol. 161, 151–164. https://doi.org/10.1046/j.1469-8137.2003.00944.x.

Ponocná, T., Spyt, B., Kaczka, R., Büntgen, U., Treml, V., 2016. Growth trends and climate responses of Norway spruce along elevational gradients in East-Central Europe. Trees 30, 1633–1646. https://doi.org/10.1007/s00468-016-1396-3.

Poorter, H., Knopf, O., Wright, I.J., Temme, A.A., Hogewoning, S.W., Graf, A., Pons, T.L., 2022. A meta-analysis of responses of C3 plants to atmospheric CO_2: dose–response curves for 85 traits ranging from the molecular to the whole-plant level. New Phytol. 233 (4), 1560–1596. https://doi.org/10.1111/nph.17802.

Prokop, O., Kolář, T., Büntgen, U., Kyncl, J., Kyncl, T., Bošeľa, M., Rybníček, M., 2016. On the palaeoclimatic potential of a millennium-long oak ring width chronology from Slovakia. Dendrochronologia 40, 93–101. https://doi.org/10.1016/j.dendro.2016.08.001.

Rajsnerová, P., Klem, K., Holub, P., Novotná, K., Večeřová, K., Kozáčiková, M., Urban, O., 2015. Morphological, biochemical and physiological traits of upper and lower canopy leaves of European beech tend to converge with increasing altitude. Tree Physiol. 35 (1), 47–60. https://doi.org/10.1093/treephys/tpu104.

Rybníček, M., Koňas, P., Kolář, T., 2010. The benefits of tree-ring curves detrending for dating archaeological wood. Geochronometria 35 (1), 85–90. https://doi.org/10.2478/v10003-010-0004-6.

Rybníček, M., Kolář, T., Ač, A., Balek, J., Koňasová, E., Trnka, M., Büntgen, U., 2021. Non-pooled oak (*Quercus* spp.) stable isotopes reveal enhanced climate sensitivity compared to ring widths. Clim. Res. 83, 27–41. https://doi.org/10.3354/cr01632.

Saurer, M., Voelker, S., 2022. Intrinsic water-use efficiency derived from stable carbon isotopes of tree-rings. In: Siegwolf, R.T.W., Brooks, J.R., Roden, J., Saurer, M. (Eds.), Stable Isotopes in Tree Rings: Inferring Physiological, Climatic and Environmental Responses. Springer International Publishing, Cham, Switzerland, pp. 481–498.

Saurer, M., Spahni, R., Frank, D.C., Joos, F., Leuenberger, M., Loader, N.J., Young, G.H., 2014. Spatial variability and temporal trends in water-use efficiency of European forests. Glob. Chang. Biol. 20 (12), 3700–3712. https://doi.org/10.1111/gcb.12717.

Seibt, U., Rajabi, A., Griffiths, H., Berry, J.A., 2008. Carbon isotopes and water use efficiency: sense and sensitivity. Oecologia 155, 441–454.

Seneviratne, S.I., Zhang, X., Adnan, M., Badi, W., Dereczynski, C., Di Luca, A., Zhou, B., 2021. Weather and climate extreme events in a changing climate. In: Masson-Delmotte, V., Zhai, P., Pirani, A., Connors, S.L., Péan, C., Berger, S. (Eds.), Climate Change 2021 – The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, pp. 1513–1766.

Šigut, L., Holišová, P., Klem, K., Šprtová, M., Calfapietra, C., Marek, M.V., Urban, O., 2015. Does long-term cultivation of saplings under elevated CO_2 concentration influence their photosynthetic response to temperature? Ann. Bot. 116 (6), 929–939. https://doi.org/10.1093/aob/mcv043.

Splechtna, B.E., Dobrys, J., Klinka, K., 2000. Tree-ring characteristics of subalpine fir (*Abies lasiocarpa* (Hook.) Nutt.) in relation to elevation and climatic fluctuations. Ann. For. Sci. 57 (2), 89–100. https://doi.org/10.1051/forest:2000105.

Strange, B.M., Monson, R.K., Szejner, P., Ehleringer, J., Hu, J., 2023. The North American Monsoon buffers forests against the ongoing megadrought in the Southwestern United States. Glob. Chang. Biol. 29, 4354–4367. https://doi.org/10.1111/gcb.16762.

Sun, X., Wang, G., Huang, M., Chang, R., Hu, Z., Song, C., Sun, J., 2020. The asynchronous response of carbon gain and water loss generate spatio-temporal pattern of WUE along elevation gradient in southwest China. J. Hydrol. 581, 124389 https://doi.org/10.1016/j.jhydrol.2019.124389.

Tetens, O., 1930. Über einige meteorologische Begriffe. Z. Geophys. 6, 297–309.

Torbenson, M.C.A., Büntgen, U., Römer, P., Urban, O., Trnka, M., Ač, A., Esper, J., 2023. Assessing earlywood-latewood proportion influence on tree-ring stable isotopes. Dendrochronologia 82, 126147. https://doi.org/10.1016/j.dendro.2023.126147.

Treydte, K., Liu, L., Padrón, R.S., Martínez-Sancho, E., Babst, F., Frank, D.C., Loader, N. J., 2024. Recent human-induced atmospheric drying across Europe unprecedented in the last 400 years. Nat. Geosci. 17, 58–65. https://doi.org/10.1038/s41561-023-01335-8.

Urban, O., 2003. Physiological impacts of elevated CO_2 concentration ranging from molecular to whole plant responses. Photosynthetica 41, 9–20. https://doi.org/10.1023/A:1025891825050.

Urban, O., Ač, A., Kolář, T., Rybníček, M., Pernicová, N., Koňasová, E., Trnka, M., Büntgen, U., 2021. The dendroclimatic value of oak stable isotopes. Dendrochronologia 65, 125804. https://doi.org/10.1016/j.dendro.2020.125804.

Vitasse, Y., Delzon, S., Bresson, C.C., Michalet, R., Kremer, A., 2009. Altitudinal differentiation in growth and phenology among populations of temperate-zone tree species growing in a common garden. Can. J. For. Res. 39 (7), 1259–1269. https://doi.org/10.1139/X09-054.

Waterhouse, J.S., Switsur, V.R., Barker, A.C., Carter, A.H.C., Hemming, D.L., Loader, N. J., Robertson, I., 2004. Northern European trees show a progressively diminishing response to increasing atmospheric carbon dioxide concentrations. Quat. Sci. Rev. 23 (7–8), 803–810. https://doi.org/10.1016/j.quascirev.2003.06.011.

Weiwei, L.U., Xinxiao, Y.U., Guodong, J.I.A., Hanzhi, L.I., Ziqiang, L.I.U., 2018. Responses of intrinsic water-use efficiency and tree growth to climate change in semi-arid areas of North China. Sci. Rep. 8 (1), 308. https://doi.org/10.1038/s41598-017-18694-z.

Wu, G., Liu, X., Chen, T., Xu, G., Wang, W., Zeng, X., Zhang, X., 2015. Elevation-dependent variations of tree growth and intrinsic water-use efficiency in Schrenk spruce (*Picea schrenkiana*) in the western Tianshan Mountainsm, China. Front. Plant Sci. 6, 309. https://doi.org/10.3389/fpls.2015.00309.

Wu, G., Liu, X., Kang, S., Chen, T., Xu, G., Zeng, X., Kang, H., 2018. Age-dependent impacts of climate change and intrinsic water-use efficiency on the growth of Schrenk spruce (*Picea schrenkiana*) in the western Tianshan Mountains, China. For. Ecol. Manag. 414, 1–14. https://doi.org/10.1016/j.foreco.2018.02.008.

Xue, B.L., Guo, Q., Otto, A., Xiao, J., Tao, S., Li, L., 2015. Global patterns, trends, and drivers of water use efficiency from 2000 to 2013. Ecosphere 6, 1–18. https://doi.org/10.1890/ES14-00416.1.

Yang, T., Yang, B., Boucher, É., Rossi, S., 2021. How did climate and CO_2 concentration affect intrinsic water-use efficiency and tree growth in a semi-arid region of China? Trees 35, 769–781. https://doi.org/10.1007/s00468-020-02075-7.

Zhang, S.B., Zhou, Z.K., Hu, H., Xu, K., Yan, N., Li, S.Y., 2005. Photosynthetic performances of *Quercus pannosa* vary with altitude in the Hengduan Mountains, southwest China. For. Ecol. Manag. 212 (1–3), 291–301. https://doi.org/10.1016/j.foreco.2005.03.031.

Zhang, J., Yang, S., Yang, S., Fan, L., Zhou, X., 2023. Spatio-temporal variations of ecosystem water use efficiency and its drivers in southwest China. Land 12 (2), 397. https://doi.org/10.3390/land12020397.