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COMMENTARY
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No Age Trends in Oak Stable Isotopes

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Key Points:
- We present and analyze 27,080 annually resolved stable carbon and oxygen measurements from living and relict oaks
- Absence of age trends in stable carbon and oxygen measurements from Czech oaks over the past 2,000 years
- Nonpooled oak stable isotope ratios are a superb paleoclimatic archive for central Europe where other tree-ring parameters fail

Supporting Information:
- Supporting Information S1
- Data S1
- Data S2

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Abstract Although the importance of stable isotope ratios in tree rings is increasing for high-resolution climate reconstructions, it is still unclear if such values exhibit age trends that require some form of standardization. Here we present 13,496 and 13,584 annually resolved and absolutely dated $\delta^{18}O$ and $\delta^{13}C$ measurements from 147 living and relict oaks (*Quercus* spp.) that grew over the past 2,000 years in the Czech Republic. In contrast to their heteroscedastic ring widths, the stable isotopes reveal constant spread versus level relationships over the trees' life span. Together with high signal strength, the absence of age-related constraints makes $\delta^{18}O$ and $\delta^{13}C$ from oak latewood alpha cellulose a superior climate proxy in regions where traditional tree-ring parameters are limited.

Plain Language Summary Tree-ring stable isotopes are important paleoclimatic archives in regions where traditional dendrochronological parameters, such as ring width and wood density, perform poorly. However, it remains debatable if isotopic ratios contain nonclimatic age trends that require some initial statistical treatment. A well-replicated compilation of annually resolved and absolutely dated stable oxygen and carbon isotope ratios in 21 living and 126 relict oaks from the Czech Republic provides unprecedented evidence to assess this biostatistical and tree physiological conundrum. Evenly distributed over the past 2,000 years, neither the 13,496 individual $\delta^{18}O$ nor the 13,584 individual $\delta^{13}C$ measurement values exhibit any detectable trend during the life span of the oaks investigated. In rejecting age-related limitations, and demonstrating strong temperature dependency, we conclude that nonpooled oak stable isotope ratios are possibly the best paleoclimatic archive for the central European lowlands and other areas where the species was most commonly used as construction timber, and where conventional tree-ring parameters often fail.

1. Background and Motivation

Considered the backbone of high-resolution paleoclimatology (St. George & Esper, 2019), and thus providing a natural context for the Anthropocene (Lewis & Maslin, 2015; Waters et al., 2016), annually resolved and absolutely dated tree-ring chronologies allow temperature or hydroclimate to be reconstructed over the past centuries to millennia. Consistent with the principle of limiting factors (Fritts, 1976), tree-ring widths (TRW) and particularly maximum latewood density from cold-moist, high-altitude/-latitude sites often reflect growing season temperature, and TRW from warm-arid sites may reveal soil moisture availability. Whereas the concept of ecological amplitude tells us that the dendroclimatological skill of forest trees generally decreases with increasing distance from species-specific distribution limits, tree-ring stable isotopes (TRSI) can exhibit strong climate signals even when the wood samples are coming from less extreme sites (Cernusak & English, 2015; Hartl-Meier et al., 2015; McCarroll & Loader, 2004; Treydte et al., 2007).

Nevertheless, it is still unclear if TRSI contain age-related trends that require some sort of statistical treatment, so-called standardization or detrending (Cook & Kairiukstis, 1990). The first indication of nonclimatic age trends in $\delta^{13}C$ was published by Freyer (1979), and later by Schleser and Jayasekera (1985). Further evidence of age trends in $\delta^{13}C$ was reported from different conifer species growing in northern Idaho, USA (Monserud & Marshall, 2001), as well as from nonpooled $\delta^{13}C$ values in oaks from different sites across central France (Etien et al., 2008). Although Gagen et al. (2007, 2008) revealed some signs of juvenile growth effects on $\delta^{13}C$ in pines from Finland, they also showed that stable tree-ring carbon isotopes were free of any coherent long-term age-related trends. Likewise, Daux et al. (2011), found no substantial age-related low-frequency behavior in the carbon isotopic signature of *Larix decidua* cellulose from the French Alps, which agrees with observations from living and relict pines from the Torneträsk region in northern Sweden (Loader, Young, McCarroll, & Wilson, 2013). Furthermore, Young et al. (2011) reported no long-term age-related trends in stable carbon and oxygen isotopes from living pines that grew under oceanic conditions at Forfjorddalen in northwestern Norway. Another case study from the UK also demonstrated the absence of age-related trends in $\delta^{18}O$ from 17 living and 32 historical oaks (Duffy et al., 2019).

In contrast, evidence for nonclimatic age-related trends in pooled $\delta^{13}C$ was revealed by 182 subfossil pines from northern Finnish Lapland (Helama et al., 2015), with comparable trends found in 76 living beech and spruce trees from seven sites across Europe (Klesse et al., 2018). The first indication of age dependency in $\delta^{18}O$ came from an annually resolved, 1,000-year-long, high-elevation juniper chronology from northern Pakistan (Treydte et al., 2006), with some additional signs of $\delta^{18}O$ age effects in oaks originating from western France (Labuhn et al., 2014). Likewise, 25 high-elevation pines from the Spanish central Pyrenees exhibit age-related changes in the spread versus level relationship of decadal $\delta^{18}O$ and $\delta^{13}C$ discrimination (Esper et al., 2010). Moreover, a diverse collection of pooled hydrogen isotope ratios (δD) in 82 subfossil oaks from Germany reveals age-dependent increases (Mayr et al., 2003).

All of the existing tree-ring stable isotope studies are, however, limited by either (i) a relatively low sample size, (ii) a restriction to only living trees, (iii) the use of pooled samples, or (iv) some combination thereof.

2. Nonpooled Tree-Ring Stable Isotopes

To better understand if oak TRSI may or may not contain age trends, and to further evaluate the findings of Duffy et al. (2019), an ideal dendroisotopic data set should consist of (i) massive sample size (>100 trees), (ii) annual isotopic ratios of individual trees (nonpooled), and (iii) evenly aged samples with different start and end dates throughout time (industrial and preindustrial material). Here we present 27,080 annually resolved and absolutely dated $\delta^{18}O$ and $\delta^{13}C$ measurements of 147 living and relict Czech oaks. After visual and statistical cross-dating, and careful splitting with a scalpel under a stereomicroscope, the latewood alpha cellulose of each tree ring was extracted, and its isotopic composition was measured (Saurer et al., 1997). We therefore followed the modified Jayme-Wise isolation method (Boettger et al., 2007). Originating from either cores or disks, the ~0.5-mm-wide, individual wood samples were packed into Teflon filter bags, and washed with 5% NaOH solution twice for 2 hr at 60 °C, followed by an additional wash with 7% $NaClO_2$ solution for another 30 hr at 60 °C. Acetic acid (99.8%) was added to the solution to keep pH 4–5. After washing, the bags with extracted cellulose were rinsed 3 times in hot distilled water (90 °C). Each sample was dried subsequently at 50 °C for 24 hr, locked in Eppendorf microtubes, and stored under dark and temperature-controlled conditions at 21 °C before analysis.

For the independent determination of carbon and oxygen isotopes, alpha cellulose samples between 0.2 and 1.0 mg were placed in tin and silver capsules, respectively. For $\delta^{13}C$ ($\delta^{18}O$), samples were combusted (pyrolyzed) to CO_2 (CO) at 960 °C (1,450 °C). Stable isotopes in the CO_2 and CO gases were then determined by a continuous flow isotope ratio mass spectrometer ISOPRIME100 (Isoprime, Manchester, UK). Prior to each set of isotopic measurements, the ion source of the mass spectrometer was centered, tuned, and tested for stability (standard deviation ≤0.04‰ on 10 pulses over three consecutive runs) and linearity (≤0.03‰/nA) over the entire range of expected ion currents obtained from the measurements of the test samples. Standard deviation was ≤0.06‰ ($\delta^{13}C$) and ≤0.10‰ ($\delta^{18}O$) on five consecutive measurements of the same alpha cellulose sample. The system was calibrated using certified reference materials with known isotopic ratios from the International Atomic Energy Agency (IAEA, Vienna, Austria). The $\delta^{13}C$ values were referenced to caffeine (IAEA-600) and graphite (USGS24). The $\delta^{18}O$ values were referenced to benzoic acid (IAEA-601 and IAEA-602). The $\delta^{13}C$ and $\delta^{18}O$ values (‰) were calculated as the deviation from the Vienna Pee Dee Belemnite and Vienna Standard Mean Ocean Water standards, respectively, according to

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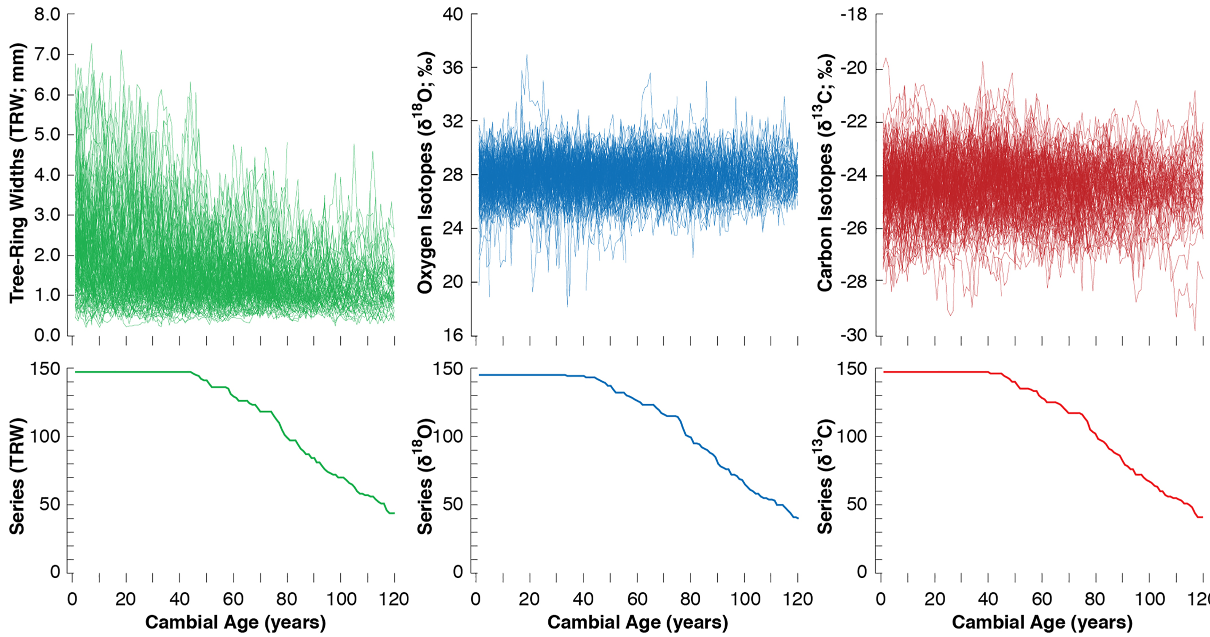

Figure 1. Age-related behavior of TRW and TRSI. The top row shows the individual raw 147 TRW and TRSI measurement series aligned by cambial age. The bottom graphs display the continuously declining sample size of the age-aligned TRW and TRSI data, ranging from 147 individual series at cambial age 1 to 40 series at cambial age 120. Carbon and oxygen isotope ratios are reported in per mil (‰) using the usual delta (δ) notation relative to the Vienna Pee Dee Belemnite ($\delta^{13}C$) and Vienna Standard Mean Ocean Water ($\delta^{18}O$) standards (Coplen, 1995).

the formula $R = (R_{sample}/R_{standard} - 1)1{,}000$, where R is the ratio of the heavy to light isotope ($^{13}C/^{12}C$, $^{18}O/^{16}O$).

Continuously covering the period from 91 BCE to 2018 CE with a mean sample size of 6.57 series (stdev = 1.06), the 13,496 $\delta^{18}O$ and 13,584 $\delta^{13}C$ values from mature *Quercus robur* and *Quercus petraea* represent a well-replicated, multimillennial-long, nonpooled TRSI data set. The mean age of the 21 living and 126 relict oaks is 105 years. Independent of their calendar date, each individual raw TRW and TRSI measurement series was aligned by cambial age (Esper et al., 2003; Figure 1). To assess possible age-related trends in TRW and TRSI, we calculated the mean and median of the age-aligned individual raw measurements and smoothed them with cubic spline functions of 50% frequency cutoff at 30 years (Cook & Peters, 1981). The resulting curves were split into three equally long, 40-year subperiods (1–40, 41–80, and 81–120 years), during which sample size declines continuously from 147 to 40 series. The mean and median, as well as the standard deviation and standard error, were calculated from the raw values for each subperiod. To the 31 $\delta^{13}C$ carbon series from living trees and historical timbers that have values after 1859 CE, we additionally applied a correction factor for atmospheric $\delta^{13}C$ depletion from anthropogenic fossil fuel emissions (McCarroll & Loader, 2004). Due to possible effects of the modern atmospheric CO_2 increase on tree physiology, the $\delta^{13}C$ values might still contain some degree of bias (McCarroll et al., 2009; Treydte et al., 2009).

In contrast to the heteroscedastic nature of the raw oak TRW measurements (Figures 1 and 2), none of their corresponding TRSI values from the same trees reveal any statistically discernible long-term trend over the first 120 years of tree growth. To corroborate our findings, we applied different methods within two Monte Carlo resampling strategies (Block-Bootstrap and Jack-Knife; Isobe et al., 1990), and used Analysis of Variance (Fisher, 1918) performed on the various time series. Only the age-aligned raw TRW measurements exhibit a significant negative trend ($p < 0.0102$). In addition, we examined the significance of each time series using a modified Mann-Kendall test (Mann, 1945), specifically adapted to autocorrelated data. Again, only the TRW data exhibit a statistically significant negative trend ($p < 0.01$). While TRW measurements decline exponentially with cambial age from ~2.0 to <0.5 mm (Figure 2), $\delta^{18}O$ exhibits negligible fluctuations ~27.5‰, and $\delta^{13}C$ remains even more stable at around −24.5‰. In contrast to TRW, TRSI does not show any statistically significant differences in their mean/median and standard error when calculated

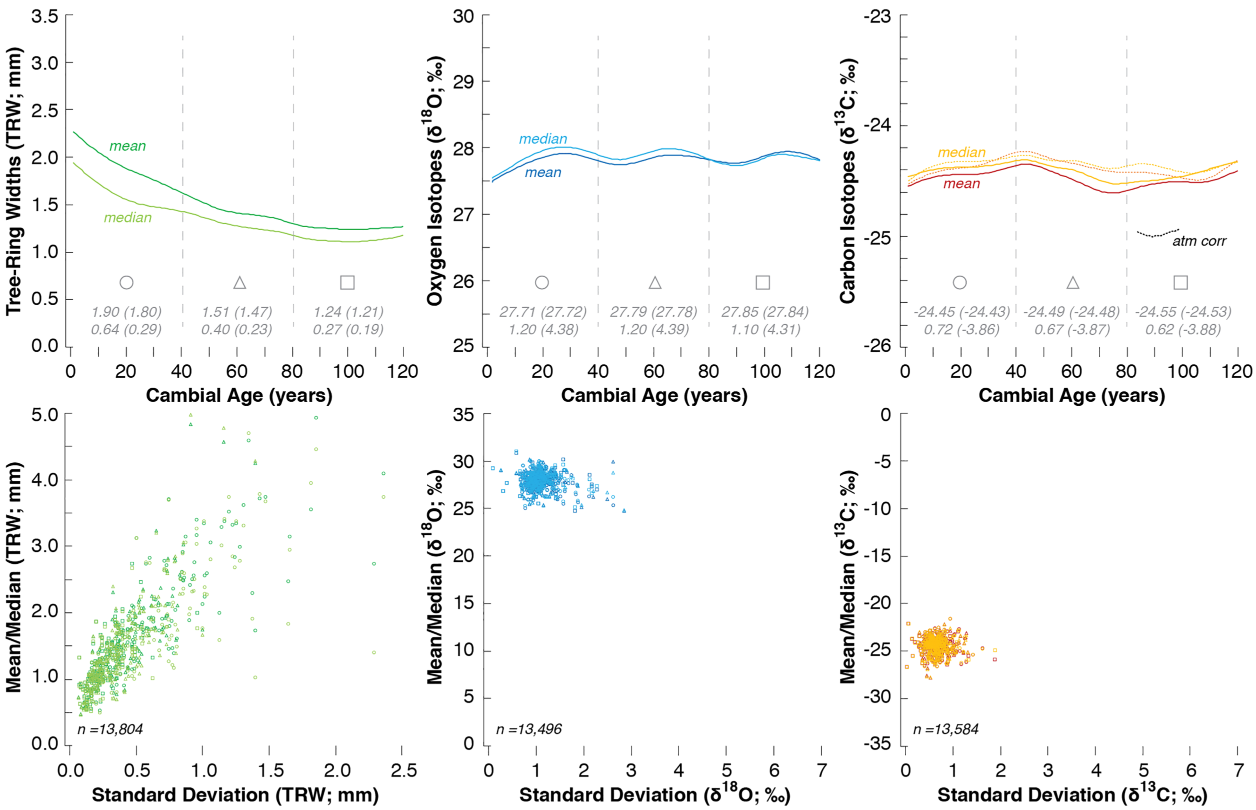

Figure 2. No age trends in TRSI. The top row shows the 30-year smoothed mean and median of the age-aligned 13,804 TRW, 13,496 oxygen, and 13,584 carbon measurements from the 147 living and relict oaks that grew during the Common Era in the Czech Republic (from left to right). Small differences in the total number of TRW and TRSI values result from insufficient wood quality and quantity that is needed for high-resolution, high-precision isotopic measurements. The lower grey values refer to the mean (median) and standard deviation (error) of the three equally long subperiods of cambial age (1–40, 41–80, and 81–120 years). The bottom part of the figure shows the relationship between the mean/median and standard deviation of the TRW and TRSI values of the 147 oaks calculated for each of the three cambial subperiods, which are displayed by the three different symbols: circles (1–40 years), triangles (41–80 years), and squares (81–120 years). Note that the data points of the TRSI from the three subperiods strongly overlap, whereas the TRW values of the subperiods scatter widely.

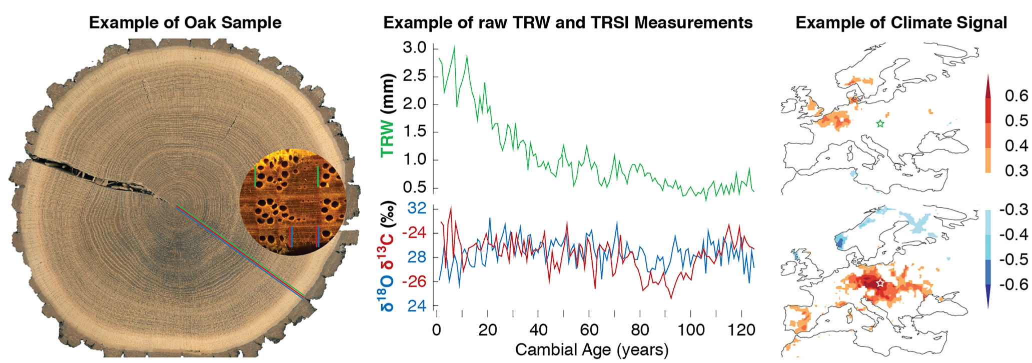

Figure 3. Sketch of the advanced paleoclimatic value of TRSI compared to TRW. Example of a polished cross section of an oak from the Czech Republic, with colored lines denoting exemplary TRW and TRSI measurement radii (green and red/blue, respectively). The magnified inset shows the anatomical structure of large earlywood vessels and homogeneous latewood fibers. The green lines mark the width of one complete oak ring, whereas the blue and red lines refer to the width of the latewood only. The middle part of the sketch is an example of raw TRW and TRSI measurement behavior from one of the 147 Czech oak samples (Kyl38). (right side) Maps compare the June–August scPDSI sensitivity of a standardized TRW chronology of 861 Czech oak TRW series after individual 100-year spline detrending against the hydroclimatic signal strength of the averaged, nonpooled, and inverse $\delta^{18}O$ and $\delta^{13}C$ values from a selection of six oaks only. Spatial field correlations from 1950 to 2017 are based on the most recent gridded 0.5° scPDSI product of the Climatic Research Unit (CRU; after van der Schrier et al., 2006).

independently over the three, 40-year-long, cambial subperiods of juvenile, mature, and adult growth (see ANOVA results in the online supporting information). Furthermore, this finding does not change when using shorter, 30-year subperiods instead. The combination of temporally stable mean/median TRSI values, and almost no variation in standard deviation, implies that the individual raw oxygen and carbon ratios are homoscedastic and therefore do not require age trend standardization/detrending. Nevertheless, any parsimonious removal of perceived nonclimatic noise, including the correction for changes in the Earth's atmospheric composition, will only further stabilize long TRSI chronologies (Helama et al., 2018), and thus ideally enhance the environmental signal of large isotopic data sets (Loader, Young, Grudd, & McCarroll, 2013). In order to reflect low-frequency information above the individual segment lengths (Cook et al., 1995), TRSI, however, do not require the application of age-related composite detrending techniques, such as the Regional Curve Standardization (Esper et al., 2003; Helama et al., 2017).

3. Discussion and Conclusions

Based on an extremely well-replicated, annually resolved, and nonpooled $\delta^{18}O$ and $\delta^{13}C$ compilation, this study emphasizes the dendroclimatological/dendroecological advantage of TRSI over TRW measurements, the latter of which not only require standardization/detrending but also possess a rather weak hydroclimatic signal (Figure 3). By contrast, the simple mean of the inverse, nondetrended $\delta^{18}O$, and $\delta^{13}C$ time series correlates highly significantly ($p < 0.001$) with June–August mean scPDSI (self-calibrated Palmer Drought Severity Index; van der Schrier et al., 2006) over much of central and eastern Europe ($r > 0.75_{1950–2017}$). The overall positive (negative) relationship between TRSI and summer temperature (hydroclimate) is in line with previous oak-based stable isotope studies from western and central Europe (Treydte et al., 2007; Etien et al., 2008; Rinne et al., 2013; Young et al., 2015; Labuhn et al., 2016). The exceptionally strong hydroclimatic signal in the TRSI of the Czech oaks likely results from a reasonably homogeneous moisture supply during their growing season when physiological processes are mainly controlled by atmospheric vapor pressure.

In re-thinking the boundaries of dendrochronology (Büntgen, 2019), our results are particularly important for the wider paleoclimatic community, because the longest, continuous TRW chronologies (Becker, 1993; Friedrich et al., 2004), and perhaps the highest density of TRW measurements from living oaks, as well as historical timbers, archeological wood, and subfossil remains come from central Europe's low-elevation oak forests (Prokop et al., 2017; Tegel et al., 2010, 2012). While oak TRW composite chronologies have been used to reconstruct hydroclimate over the past millennia with reasonable success (Büntgen et al., 2010, 2011; Cook et al., 2015; Dobrovolný et al., 2018), the superb signal strength of their nonpooled TRSI—individual or combined—suggests a clear paleoclimatic improvement for those regions where TRW generally fails (Figure 3). TRSI may even help resolving difficult dendroarcheological/dendrohistorical dating issues (Loader et al., 2019). The existence of ultralong oak TRW chronologies in France, Germany, and the British Isles foster great potential for TRSI-based warm-season hydroclimatic reconstructions to cover much of the Holocene at annual resolution. Beyond the enhancement of climate reconstructions, TRSI—supplementary rather than exclusively—will further improve our knowledge about the direct and indirect responses of forest ecosystems in the Anthropocene (Cernusak & English, 2015), with consequences as far reaching as the disruption of the global carbon cycle and possible species extinction.

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