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Assessing earlywood-latewood proportion influence on tree-ring stable isotopes

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ABSTRACT

Tree-ring stable isotopes are typically measured in latewood cellulose to mitigate potential carry-over effects from previous year storage pools. The isotopic composition of individual tree-ring segments is thought to include considerable intra-annual variability. This sampling strategy may be complicated by steep intra-annual isotope gradients that can rival the inter-annual variability, however. Consistent sampling of latewood material may not always be possible due to low sample availability or high prevalence of narrow rings or low amounts of latewood because of species-specific changes in ring width. Therefore, years that contain samples with higher portions of non-latewood (earlywood) material may influence the final chronology of isotopic variability. Here, we analyze the potential influence that changing earlywood and latewood components of individual tree rings can have on stable carbon and oxygen records from *Quercus* spp. and *Pinus heldreichii* chronologies. Analysis of stable isotopes in oak tree rings with varying amounts of latewood show no statistically significant differences in the range of isotopic composition, nor any major differences when considering the same calendric year. Similar results were found for the pine data, when comparing stable isotope measurements with earlywood-to-latewood ratio and maximum density. We argue that this simple approach should be applied to any long-term tree-ring stable isotope record in order to provide a better understanding of the potential biases that could arise from previously recorded intra-annual variability in the wood.

1. Introduction

Tree-ring stables isotopes (TRSIs) are increasingly used for climate reconstructions (e.g., Labuhn et al., 2016; Nguyen et al., 2022; Freund et al., 2023) due to their ability to record stronger and/or different signals compared to traditional tree-ring parameters, such as total ring-width (TRW) (Rybníček et al., 2021). However, the physiological processes that influence carbon ($\delta^{13}C$) and oxygen ($\delta^{18}O$) isotopes are complex (McCarroll and Loader, 2004; Belmecheri et al., 2018), and a diverse range of climate signals have been extracted (Leavitt, 2010). The various approaches to measuring isotopic composition, including different sampling techniques (Leavitt and Szejner, 2022), material selection (e.g., bulk wood, cellulose, or lignin; Loader et al., 2003), and pooling or non-pooling of material (Leavitt, 2008), can influence the interpretation of the environmental signals expressed in the TRSIs. Other factors that can skew comparisons between TRSI records include differences in low-frequency variability (Esper et al., 2010) and age-related trends (e.g., Arosio et al., 2020), but even for these characteristics some studies on the same species and from the same region have resulted in different conclusions (Gagen et al., 2008; Helema et al., 2015; Torbenson et al., 2022).

Another potential complication for interpreting TRSI chronologies and their climate signals possibly emerges from intra-annual changes in wood composition. Most tree species from the extra-tropics display shifts

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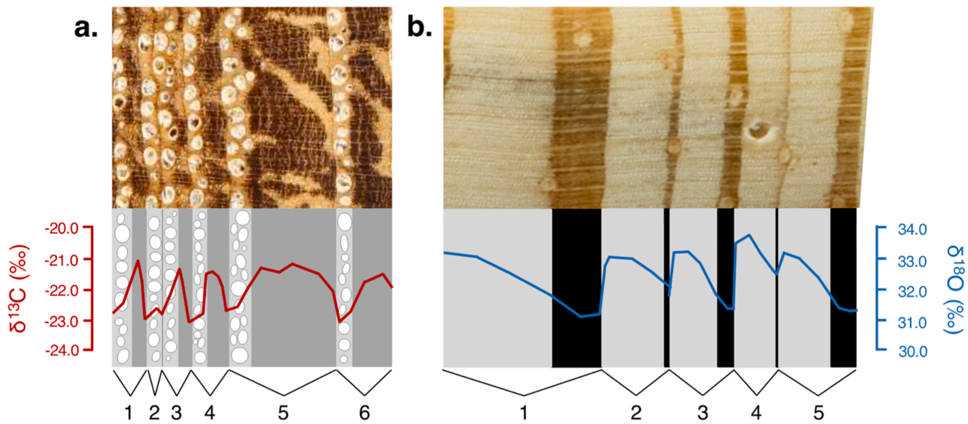

Fig. 1. Tree-ring sequences from the (a) *Quercus* and (b) *Pinus* genera. Note the interannual variability in earlywood (EW) and latewood (LW) width, relative to the total ring width (TRW). The second tree ring of oak (from the left) represents little-to-no LW material to sample for isotopic studies. Conversely, the third and fourth pine rings are of similar TRW, but the amount of latewood differs considerably. The intra-annual fluctuations of $\delta^{13}C$ and $\delta^{18}O$ plotted on top of the ring components are theoretical (based on literature values (Ogle and McCormac, 1994; Li et al., 2011)) and only shown for visualization.

in density within the wood layer formed during the growing season – something that is foundational to the identification of annual rings (Schweingruber, 1993). Both conifer and deciduous species are known to form earlywood (EW) and latewood (LW) components (Fig. 1), with varying independent variability across space and species boundaries (Torbenson et al., 2016). If isotopic composition is related to wood density or the EW/LW ratio (independent of a common relationship with climate), extracting cellulose from entire rings (or pre-determined subdivision of rings that are not always available for the full record) may introduce a bias. Some isotopic chronologies previously produced contain gaps because inadequate amounts of latewood were present for some year (e.g., Esper et al., 2017).

Earlier TRSI studies demonstrated a high variability of the isotopic signal – not only between different tree species but also within individual trees and even tree rings (Wilson and Grinsted, 1977, 1978; Leavitt and Long, 1986). Ogle and McCormac (1994) reported a depletion of $\delta^{13}C$ in the earlywood of Northern Irish *Quercus robur*; however, a subsequent study on *Quercus petraea* (and other broadleaf species) from Germany indicated that the lowest $\delta^{13}C$ values can be found in the latewood (Helle and Schleser, 2004). Conifers have displayed varying magnitudes of shifts in isotopic variability (e.g., Roden et al., 2009; Schubert and Jahren, 2015; Li et al., 2023) but these changes are difficult to generalize as even within-species differences were described, possibly due to site-specific climatic conditions (e.g., Szejner et al., 2016), and such differences can even change through time in different climatic conditions (Kimak and Leuenberger, 2015). Sampling and extraction techniques of TRSIs, as well as the accuracy and precision of their measurements, may thus play a critical role in the diverse results of older works. For instance, lignin-to-cellulose ratio differs between earlywood and latewood (Kagawa and Battipaglia, 2022) and cellulose exhibits distinct isotopic compositions compared to lignin (Loader et al., 2003). More recent studies from various continents and environments have utilized technological advances to study the isotopic composition throughout the wood of a single year (e.g., Nabeshima et al., 2018; Xu et al., 2020; Giraldo et al., 2022; Li et al., 2023). Several reasons for these intra-annual changes may be hypothesized (such as the timing (e.g., Szejner et al., 2016) and/or storage (e.g., Helle and Schleser, 2004) of photoassimilates), but the climatic drivers Nonetheless, common to these studies are non-stationary isotopic ratios throughout individual tree rings.

Because evidence from both broadleaved (e.g., Helle and Schleser, 2004; Nabeshima et al., 2018) and coniferous (e.g., Fu et al., 2017; Xu et al., 2020) tree species reveals systematic shifts in isotopic ratios from earlywood to latewood, testing what effect, if any, the EW-LW proportion can have on TRSIs, and subsequent chronologies, must be considered necessary. Changing proportions of EW-LW through a tree's lifespan could in theory produce age-related trends in stable isotope series if there is a bias in isotopic ratios related to wood component type. Here, we assess if rings with larger EW portions differ significantly in their isotopic composition from rings with a greater LW amount, by analyzing TRSI and ring-width data from the largest available, non-pooled and millennia-long deciduous and coniferous datasets. The outlined post hoc test represents a simple analysis that can be applied to other existing datasets.

2. Materials and methods

Tree-ring samples from 145 central European oaks (*Quercus robur* and *Q. petraea*) and 26 pines (*Pinus heldreichii*) from Greece were studied. The oak samples comprise of material from living, historical, archaeological, and subfossil wood. The vast majority of these samples come from the Czech Republic, with a small portion of earlier archaeological material from southeastern Germany (Büntgen et al., 2021), from elevations spanning 170–500 m a.s.l. Despite being collected from different locations, all TRW series cross-date with each other and the trees are assumed to have grown under similar conditions. The growing season starts in mid-April to May (Kuster et al., 2014), and coincides with the beginning of the wettest months in the region. A previous study concluded that differences between sampling locations, species, and within-site conditions (such as elevation) play a limited role in the general characteristics of $\delta^{13}C$ and $\delta^{18}O$ variability for living trees (Urban et al., 2021). The pine material is relict wood from a single site (Mt. Smolikas, northwestern Greece), with all samples collected from above 2000 m in elevation (Klippel et al., 2017). Cambial activity in *P. heldreichii* begins in early June (Ivanova et al., 2013) and the growing season is characterized by warm and dry Mediterranean conditions.

Annually resolved TRW, $\delta^{13}C$, and $\delta^{18}O$ measurements were available for both collections. In addition, maximum latewood density (MXD), EW width, and LW width measurements were available for the pine samples. Here, EW and LW widths were produced from the density measurements (using a 50% threshold of annual density as the EW-LW boundary). Detailed descriptions of the data and methodologies to produce the measurements are provided in Klippel et al. (2017), Büntgen et al. (2020), Esper et al. (2020), Urban et al. (2021), and Römer et al. (2023). EW percentage was calculated as EW width divided by TRW. Locally absent rings were excluded from the analysis. In total, 14, 917 oak rings and 4773 pine rings, for which all tree-ring parameters are available, were analyzed. Raw $\delta^{13}C$ and $\delta^{18}O$ measurements were considered, as well as rescaled values of the same isotope series (min--max normalization).

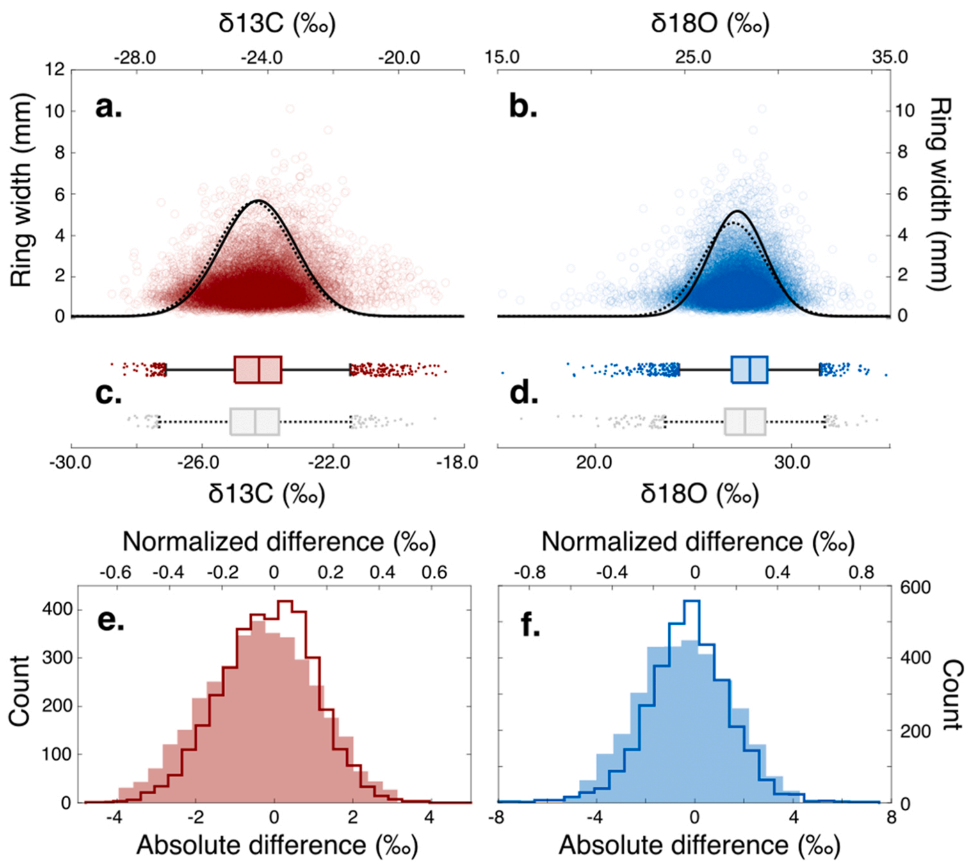

Fig. 2. Range of $\delta^{13}C$ (a) and $\delta^{18}O$ (b) values against raw TRW values in central European oaks. Normal distributions fitted to the narrow (dashed) and non-narrow rings (solid). Boxplots of $\delta^{13}C$ (c) and $\delta^{18}O$ (d) values in narrow (grey) and non-narrow (red/blue) rings. Distribution of differences between $\delta^{13}C$ (e) / $\delta^{18}O$ (f) of narrow rings and the mean of non-narrow rings for the same calendar year. Lines indicate absolute differences and shaded areas differences for the normalized stable isotope series.

Oak rings were divided into two groups depending on raw TRW (</≥1 mm). Although somewhat approximate, it is our experience that rings below this threshold often lack enough latewood to provide sufficient material for cellulose extraction - the target material for TRSI measurements in deciduous angiosperms (Urban et al., 2021). The groupings were also tested using 0.8 and 1.2 mm thresholds. Values of $\delta^{13}C$ and $\delta^{18}O$ measured from narrow rings (and therefore assumed to include EW material) were compared with those of non-narrow rings (for which the material is assumed to contain no EW). This comparison was done for the full dataset to investigate if there are differences in the range of TRSI values for rings of varying widths. The distributions of $\delta^{13}C$ and $\delta^{18}O$ values were fitted to both the narrow and non-narrow groups, and a Kolmogorov-Smirnov (KS; Smirnov, 1948; Simard and L'Ecuyer, 2011) test was applied to assess differences. To test the impact such differences can have on individual chronology years, the isotopic values of any ring < 1 mm in TRW were compared to the mean isotopic values of rings > 1 mm in TRW for the same calendar year. Years for which only narrow rings were present were excluded from the analysis. For the pine data, EW percentages were calculated for each ring, allowing for a continuous comparison rather than the binary narrow vs. non-narrow rings for the oaks.

3. Results and discussion

3.1. Stable isotopes in narrow vs. non-narrow oak growth rings

Of the 14,917 oak rings, 3674 (24.6%) were considered narrow (<1 mm). The KS tests indicate no significant difference in the distributions of either stable isotope for the narrow vs. non-narrow group. No significant differences in mean or median were recorded (Fig. 2). 88 of the narrow rings were formed during years for which no non-narrow rings were recorded (representing a total of 13 different years). The distribution of differences between $\delta^{13}C$ values in narrow and non-narrow rings is centered around zero and normally distributed for the remaining 3674 rings. The same is observed for $\delta^{18}O$ (Fig. 2**e-f**). Absolute and normalized differences display similar patterns. Narrower (0.8 mm) and wider (1.2 mm) thresholds for EW influence do not yield different results. Additionally, the same procedure performed only on samples ending prior to 1850 ($n = 101$) do not produce shifts in the distributions. Therefore, the so-called Suess effect (Keeling et al., 2017) is unlikely to impact these results.

Our findings suggest that there is no significant bias ($p > 0.05$; KS test) in the central European oak TRSI series (either in $\delta^{13}C$ or $\delta^{18}O$) stemming from TRW or varying earlywood portions. Narrow rings (and by extension, rings with lower amounts of LW material available for isotopic analysis) do not display statistically different TRSI values from non-narrow rings of the same year (Fig. 2c,f). Although there are some limitations when comparing these results to other studies (e.g., previous studies have explicitly made several measurements within one individual ring), we argue that published reconstructions based on these TRSIs (i.e., Büntgen et al., 2021; Torbenson et al., 2023) are not affected by intra-annual fluctuations in a meaningful way – at least not due to the amount of LW material sampled.

3.2. EW percentage and stable isotopes in pine

The mean percentage of EW in the 4773 pine rings is 76%, with a range from 23% to 95%. The vast majority of rings, however, contained

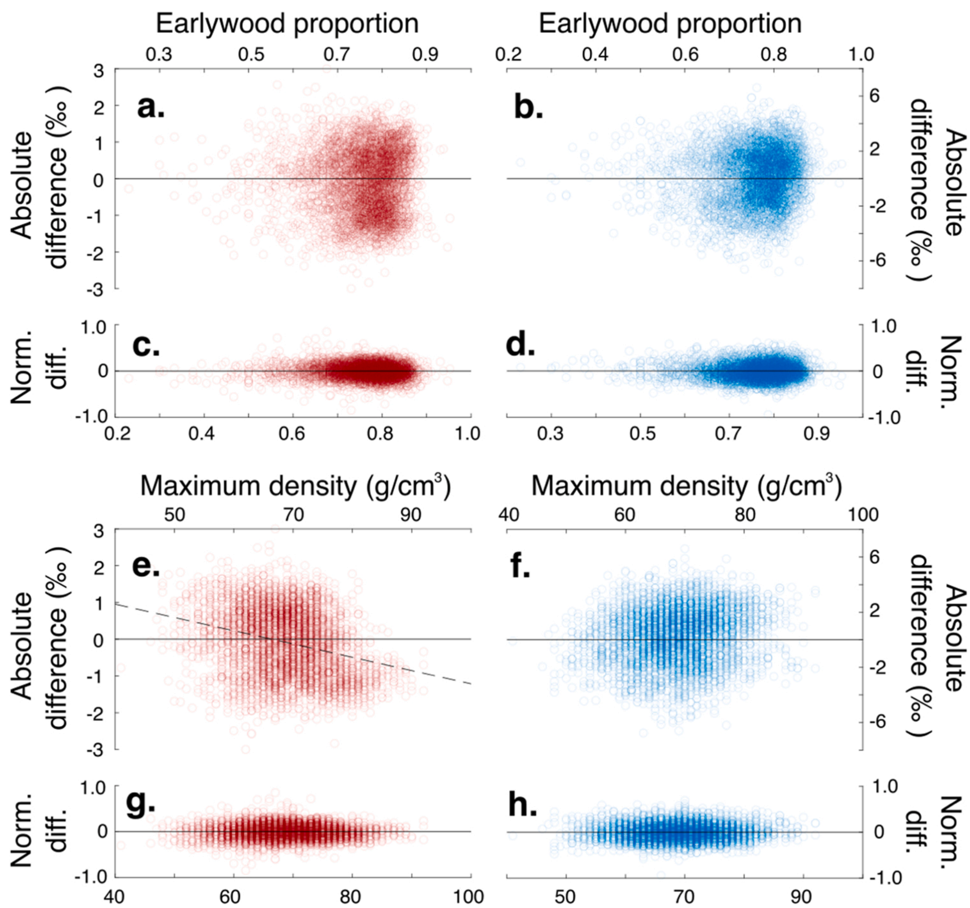

Fig. 3. Differences in $\delta^{13}C$ (a) and $\delta^{18}O$ (b) in relation to earlywood proportion and maximum density (e,f) in 26 *P. heldreichii* samples. (c,d and g,h) Same as for a,b,e, f but for normalized stable isotope series. Dashed line in (e) indicates a linear regression of r = −0.283.

EW between 62% and 85% corresponding to the 5th and 95th percentiles, respectively. No significant relationship between EW proportion and same-year isotopic composition was recorded for either stable isotope (Fig. 3). There appears to be a weak relationship between $\delta^{13}C$ and MXD, with more depleted stable carbon recorded in higher densities ($r^2 = 0.08$, Fig. 3e). However, when the $\delta^{13}C$ data is normalized and compared as departures from the individual tree mean, no such relationship is present (Fig. 3g). The difference may be caused by the high inter-tree variability in mean stable isotopic composition, as has been reported in previous TRSI studies (e.g., Leavitt, 2010). No statistically significant relationship is recorded for $\delta^{18}O$ (Fig. 3f,h).

3.3. Synthesis and outlook

The analysis presented here is not intended to be a substitute for studies on intra-annual variability of the isotopic composition in tree rings. The hypothesis tested would not have been formulated without the results from studies that focus on high-resolution measurements of $\delta^{13}C$ and $\delta^{18}O$ (e.g., Szejner et al., 2016; Li et al., 2023). There is, however, a significant temporal and sample size gap between studies on modern samples and the data underlying millennia-long climate reconstructions. Our results highlight the need to bridge that gap. The potential biases that can arise from intra-annual variability in TRSIs need to be considered – if nothing else than for producing more robust uncertainty estimates accompanying reconstructions.

Neither the oaks nor pines analyzed here record such biases. These results indicate that the intra-annual fluctuations of the isotopic ratios in tree rings impact the final multi-sample predictor chronologies. Our study is far from exhaustive, especially for the pines, and future studies that systematically compare intra-annual with full ring measurements on the same material may provide more robust conclusions. Ultimately, the underlying processes that cause within-ring fluctuations in TRSI values will only be understood through further intra-annual measurements. However, because of the labor and cost involved in producing such data, the number of datasets that have adequate sample sizes to fully assess the potential influence is likely to remain low for the foreseeable future. The high variability of statistical characteristics and climate signals exhibited by TRSIs in the literature on different species and locations would suggest that our results cannot be assumed to hold true universally. We suggest that obtaining EW and LW widths of samples processed for TRSI measurements should be prioritized in future studies to allow for a basic, yet robust, assessment of potential influences of tree ring component (without necessarily requiring intra-annual measurements). Of special interest are stable isotope series that display age-related trend, as such trend could potentially be related to the diminishing raw ring-width as the tree ages (or the ratio between wood components). Additionally, collections for which ring-width and stable isotope variability share a strong common climatic forcing may also yield different results. Therefore, reporting on the relationship between TRSI values and the amount of EW and LW can be an important metric to understand potential uncertainties previously unaccounted for in dendroclimatic reconstructions – especially for data that has yet to be made public.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Jan Esper reports financial support was provided by German Research Foundation. Miroslav Trnka reports financial support was provided by

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Data Availability

The majority of data is public and the rest (pine) will be made available shortly.

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