More than climate: Hydrogen isotope ratios in tree rings as novel plant physiological indicator for stress conditions

Marco M. Lehmann a,b, Valentina Vitali a, Philipp Schuler a, Markus Leuenberger b, Matthias Saurer a

A B S T R A C T

The analysis of stable carbon and oxygen isotopes in tree-rings is a widely applied tool which allows to retrieve information about past climatic conditions, as well as tree physiological responses to environmental changes. This is based on well-established mechanistic models and firm statistical relationships with climate variables. In contrast, the hydrogen isotopic signature (δ2H) of tree-rings has been reported to be poorly correlated to climate or difficult to explain, and as a consequence, hydrogen isotopes are far less utilized. However, recent plant-physiological experiments have highlighted the role of autotrophic versus heterotrophic processes affecting δ2H values, i.e. use of fresh assimilates versus stored carbohydrates, and have much improved our understanding of the role of post-photosynthetic δ2H-fractionation. Using unpublished and literature δ2H data of tree-ring cellulose (δ2Hc) of 5 study sites in Europe and Asia, we systematically investigated the relationships between δ2Hc and tree-ring width (TRW), which, in contrast to previous research, could now be explained through post-photosynthetic δ2H-fractionation. In most cases, these relationships were found to be negative (r 2 = 0.23 to 0.51, all P < 0.05) when the main growth limiting factors are precipitation and light, while in temperature-limited sites we observed a positive trend (r 2 = 0.14, P < 0.05). Our results suggest that, under stress conditions, trees use a surplus of carbon from reserves for wood formation. Therefore, in combination with TRW chronologies, δ2Hc may allow to infer about physiological information on stressful time periods independently of biotic and abiotic origin. Here, we discuss implications of these findings for tree-ring research, summarize them in a conceptual framework and suggest future research directions.

1. Introduction

Stable isotopes in tree rings are successfully used in climate and environmental research for the reconstruction of past climatic conditions, as well as to deepen our understanding of physiological responses of trees to environmental changes (McCarroll and Loader, 2004). Carbon and oxygen isotope ratios can be retrieved from cellulose stored in tree rings at annual and intra-annual resolution, which allows the creation of large-scale networks with high temporal resolution (Treydte et al., 2007; Saurer et al., 2014). Oxygen isotope ratios are often used to retrieve information about past changes of precipitation or atmospheric circulation patterns (Young et al., 2015), while carbon isotopes are used for reconstruction of drought, sunshine duration or estimation of intrinsic water-use efficiency (Leavitt and Long, 1988; Saurer et al., 2004). In contrast, analysis of hydrogen isotopes, also available in tree-ring cellulose molecules (δ2Hc) were rarely applied in recent dendroecological research (Voelker et al., 2014; Nakatsuka et al., 2020). The lack of interest in δ2Hc is partially caused by analytical issues with OH-groups in cellulose, where part of the hydrogen atoms exchange with OH-groups in cellulose, where part of the hydrogen atoms continue to exchange with surrounding moisture sources (DeNiro, 1981). This bias needs to be removed before inferring on the information about climatic conditions or physiological responses that remains in the so-called carbon-bound (non-exchangeable) H of the cellulose molecules. This can be done by nitration of the cellulose molecule (Green, 1963; Boettger et al., 2007). Alternatively, the cellulose OH-groups can be equilibrated with water vapour of a known isotopic composition, allowing for correction of δ2Hc results (Filot et al., 2006; Sauer et al., 2009; Wassenaar et al., 2015). On the other hand, although temperature conditions are reflected in δ2Hc on a global scale (Gray and Song, 1984), rather poor climate information has been observed in annual δ2Hc.
chronologies (Pendall, 2006; Waterhouse et al., 2002; Loader et al., 2008; Boettger et al., 2014) and this is most likely why hydrogen isotopes in tree rings are not commonly applied. This lack of a clear δ2H-climate correlations is surprising. In water pools, isotopes of oxygen (δ18O) and hydrogen (δ2H) are commonly linked by isotope fractionations in the hydrological cycle in response to environmental conditions (Dansgaard, 1964; Brooks et al., 2010). Therefore, assuming a constant biochemical isotope fractionation, one could expect that the isotopic relationship between both elements and thereby the climatic and physiological information to be preserved in the cellulose. This concept generally holds true for δ18O, where, after an initial biochemical isotope fractionation of ca. 27‰ and a set of post-photosynthetic isotope fractionations, the soil water and the leaf evaporation water signals are imprinted on tree-ring cellulose (Sternberg, 2009). On the contrary, this concept does not hold for δ2H, as isotope fractionations between water and sugars before and during cellulose synthesis are more variable and less constant (Yakir and DeNiro, 1990).

Recent research indeed indicates that δ2H values in plant carbohydrates such as photosynthetic assimilates and cellulose are influenced by strong isotope fractionations during multiple enzymatic reactions (Cormier et al., 2018; Sanchez-Bragado et al., 2019). Light leads to typically more δ2H-depleted cellulose in autotrophic than in heterotrophic plant tissues (Yakir and DeNiro, 1990; Sanchez-Bragado et al., 2019). This effect is primarily based on the light-dependent reduction of NADP to NADPH during water photosynthesis (Cormier et al., 2018), causing a δ2H-depletion in sugar molecules compared to leaf water during re- actions shortly after CO2 fixation. Cormier et al. (2018) showed that the initial biochemical δ2H-fractionations are relatively constant, but are sensitive to very low-light or low-CO2 conditions. During translocation from source to sink tissues, the δ2H values of leaf assimilates is altered by post-photosynthetic isotope fractionations. In heterotrophic tissues, carbon-bound sugars-H can exchange with water-H in various enzymatic reactions or with H derived from the hypothetically δ2H-enriched NADPH of the oxidative pentose-phosphate pathway. The partial loss of the leaf water signal and thus of the initial assimilates δ2H-depletion before tree-ring cellulose synthesis have been previously observed and explained by isotope fractionation models (Rodenhon et al., 2000). However, a growing body of evidence suggests that the utilization of carbon reserves causes an additional δ2H-enrichment in leaf and tree-ring cellulose. For instance, Kimak et al. (2015) showed that leaf cellulose of deciduous tree species is δ2H-enriched in the early growing season, when supply of fresh assimilates via photosynthetic activity is still low. Also intra-annual variations in tree-rings have often been observed, with early wood being more δ2H-enriched compared to late wood of the same year (Epstein and Yapp, 1976; Kimak, 2015; Nabeshima et al., 2018). This suggests that early wood cellulose is composed of δ2H-enriched carbohydrates derived from storage pools or that post-photosynthetic isotope fractionation change during the growing season. The idea of heterotrophic δ2H-enriched carbon storage pools is also supported by seasonal δ2H variations in lipids (Newberry et al., 2015) and by the δ2H-enrichment in starch-accumulating grain compared to other plant organs in wheat (Sanchez-Bragado et al., 2019). While these findings certainly question the use of δ2H in tree rings as a straightforward proxy for climate, here, we propose that this opens new avenues for its uses as a plant physiological indicator.

2. Material and methods

For our analysis, we used δ2Hc and tree-ring width (TRW) chronologies from five sites in Europe and Asia. The sites differed strongly in their conditions in terms of time period, tree species, average growth, region and climate (Table 1). Three out of five sites are sourced from a literature review: China (Liu et al., 2015), Germany (Lipp et al., 1993), and India (Ramesh et al., 1985). For all literature study sites, δ2Hc values are derived from the analysis of cellulose nitrate. The δ2Hc and TRW data from the other two sites, i.e. Switzerland and Norway, are part of a tree-ring isotope network (Isonet). Please see Treu and et al. (2007) for further details on the correlations of carbon and oxygen isotopes with climate shown by the Isonet network. TRW of both Isonet sites has been determined by standard dendrochronological methods. For hydrogen isotope analysis, cellulose was extracted from pooled annual rings (Norway) or from late wood (Switzerland) of four trees per site (Loader et al., 1997; Boettger et al., 2007). For the Norwegian site, the cellulose was nitrated (Green, 1963; Boettger et al., 2007), packed in silver capsules, and δ2Hc measured with a Thermal Combustion / Elemental analyser (TC/EA) coupled to a Delta PlusXL IRMS (both Thermo-Finnigan, Bremen, Germany) in the Dating Laboratory, University of Helsinki, Finland (Hiljasvuori and Berninger, 2010). For the Swiss site, the extracted cellulose was measured with an on-line equilibration technique (Filot et al., 2006). In brief, cellulose in silver capsules was equilibrated at 110 °C with water vapour of known isotopic composition for 600 s and δ2Hc measured with a TC/EA coupled to a Delta PlusXL IRMS (both Thermo-Finnigan, Bremen, Germany) in the laboratory of Climate and Environmental Physics, University of Bern, Switzerland.

δ2Hc values of all five sites are referenced to the international standard Vienna Standard Mean Ocean Water (VSMOW). All statistical analyses were performed in R version 4.0.0 (R Core Team, 2020).

3. Results and discussion

The chronologies from the Swiss oak trees (Quercus petraea) are a striking example of the strong relationship between δ2Hc and TRW and of the non-climatic information in the hydrogen isotopic composition (Fig.1, Table 1). Although available for more than 15 years, the δ2Hc

Table 1

<table>
<thead>
<tr>
<th>Source</th>
<th>Country</th>
<th>Species</th>
<th>Compound</th>
<th>Coordinates N E</th>
<th>Time period</th>
<th>MAT (°C)</th>
<th>MAP (mm)</th>
<th>Eleva. (m asl)</th>
<th>No. δ2Hc / TRW</th>
<th>δ2Hc (‰)</th>
<th>TRW (mm)</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ramesh et al., 1985</td>
<td>India</td>
<td>Abies pindrow</td>
<td>Whole ring cellulose</td>
<td>34.04 74.25</td>
<td>1901–1932</td>
<td>15.2</td>
<td>380</td>
<td>2650</td>
<td>2/2</td>
<td>−47.1 ± 15.2</td>
<td>5.9 ± 15.2</td>
<td>↗</td>
</tr>
<tr>
<td>Liu et al., 2015</td>
<td>China</td>
<td>Picea crassifolia</td>
<td>Whole ring cellulose</td>
<td>35.52 104.09</td>
<td>1954–2012</td>
<td>6.8</td>
<td>386</td>
<td>2400</td>
<td>5/27</td>
<td>−44.9 ± 15.1</td>
<td>1.2 ± 6.6</td>
<td>↗</td>
</tr>
<tr>
<td>Lipp et al., 1993</td>
<td>Germany</td>
<td>Picea abies</td>
<td>Latewood cellulose</td>
<td>49.3 10.27</td>
<td>1901–1980</td>
<td>9.2</td>
<td>700</td>
<td>330</td>
<td>5/5</td>
<td>−59.7 ± 8.5</td>
<td>1.8 ± 0.6</td>
<td>↗</td>
</tr>
<tr>
<td>Isonet</td>
<td>Switzerland</td>
<td>Quercus petraea</td>
<td>Latewood cellulose</td>
<td>46.35 8.6</td>
<td>1901–2001</td>
<td>6.8</td>
<td>1500</td>
<td>900</td>
<td>4/33</td>
<td>−51.2 ± 8.9</td>
<td>0.2 ± 0.2</td>
<td>↗</td>
</tr>
<tr>
<td>Isonet</td>
<td>Norway</td>
<td>Pinus sylvestris</td>
<td>Whole ring cellulose</td>
<td>62 12.18</td>
<td>1901–2003</td>
<td>1.5</td>
<td>575</td>
<td>800</td>
<td>4/45</td>
<td>−94.3 ± 8.9</td>
<td>0.5 ± 0.5</td>
<td>↗</td>
</tr>
</tbody>
</table>

* Unpublished δ2Hc data from the Isonet tree-ring network.
with the new findings on records of the Isonet project were not published due to lack of significant correlations to climate and problems with their interpretation. However, with the new findings on H-fractionation in mind, the influence of growth conditions on δ²H is giving a new prospective for interpretation. As an example, the extremely low δ²H value in 1961 was synchronised with extremely high tree-ring growth in the same year (Fig. 1A, B) and this opposite pattern was also confirmed for the full chronologies, as δ²H and TRW chronologies overall showed a significant negative relationship ($r^2 = 0.33$, $P < 0.05$; Fig. 1C, Table 1). In this specific case, where we are considering a high mountain protected forest, a possible cause of this sudden growth increase could be a release effect connected to a reduction in competition due to tree-fall, stand damages by stone-fall or snow avalanche, which are common in the area. Therefore, in this cool and wet site, we expect light competition or stand density to be the growth limiting factor. It might therefore be possible to better understand and identify past disturbance events based on such combined analysis.

To our knowledge, δ²H and TRW chronologies have never been systematically compared. Nonetheless, we found three additional studies (Ramesh et al., 1985; Lipp et al., 1993; Liu et al., 2015), reporting both δ²H and TRW data for conifer tree species in temperate sites where growth was limited by precipitation (380–700 mm yr⁻¹; China, Germany, India). Even though varying widely in site-specific conditions and species, the re-analysis showed a consistent negative δ²H–TRW correlation (Fig. 1C, Table 1, $r^2 = 0.23–0.51$, $P < 0.05$). These results indicate that environmental drivers of growth and H-fractionations are likely connected to a certain degree. However, a universal δ²H–TRW relationship is certainly not expected. In fact, for the pine trees in Norway we found a weak positive δ²H–TRW relationship ($r^2 = 0.14$, $P < 0.05$). Here, in contrast to the other sites, the consistently narrow TRW and the stand climatic conditions (Table 1) indicate that temperature was the growth-limiting factor.

We propose the following conceptual framework to disentangle the impact of climatic conditions and storage use on δ²H and TRW (Fig. 2), as a tool for retrospective assessment of changes in physiological processes. We can follow how the variation of δ²H values (black dashed arrow) are influenced by climatic and hydrological processes, as warm and dry conditions result in a δ²H-enrichment in hydrological components (blue arrow) such as the source water (precipitation and thus soil water) and of the leaf water used for biosynthesis of carbohydrates. However, the δ²H signal in water is not unilaterally transferred to the tree-ring cellulose. Where a “1:1 signal transfer” would reflect a constant biochemical shift (green solid arrow), a deviation from the diagonal indicates further δ²H modification by plant physiological mechanisms (grey arrows). Here, environmental conditions such as drought stress or light availability may drive the proportional use of δ²H-enriched sugars derived from heterotrophic carbon storage versus δ²H-depleted photosynthetic assimilates for growth, varying the δ²H-fractionation between water and cellulose (green dashed arrows). Further, a direct relationship to small tree rings is expected as a higher proportion of storage resources are likely used under stressful conditions. Thus, if climatic-hydrological effects are low or can be excluded (e.g. weak δ²H–δ¹⁸O relationships have been observed for tree-ring cellulose in Switzerland and Norway; both $r^2 < 0.09$, data not shown), δ²H could indicate the carbon source (storage vs. assimilates) that has been preferentially used for growth.

In conclusion, the new knowledge on δ²H-isotope fractions and our proposed conceptual framework described above and summarized in Fig. 2 should be helpful to decipher the information on physiological-
Fig. 2. Conceptual framework to disentangle the impact of climatic conditions and storage use on δ²H and TRW (Fig. 2), as a tool for retrospective assessment of changes in physiological processes. The known evaporative δ²H enrichment trend in hydrological components such as precipitation and leaf water (blue arrow) with increasingly warmer and drier conditions is transferred to the cellulose (black dashed arrow) forming the annual tree-ring. Under constant post-photosynthetic δ²H-fractionation (green solid arrow), the climatic signal in δ²H of the hydrological components would be unilaterally transferred to tree-ring cellulose. However, post-photosynthetic δ²H-fractions between the hydrological components and cellulose likely depend on carbon resources usage or availability (green dashed arrows), e.g., the proportional use of either storage or new assimilate for growth, causing variations in δ²H of tree-ring cellulose (grey arrows). Hence, a high proportion of stored carbohydrates for growth may often be related to narrow tree-rings, which results in a negative relationship with δ²H values of cellulose (narrow TRW ↔ high δ²H and vice versa) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Important environmental interactions stored in δ²Hc, and consequently, for the reconstruction of trees carbon use strategies. We advocate against an isolated interpretation of δ²Hc; or simple δ²Hc–climate correlations due to the strong physiological and metabolic interference, rather we suggest an integrated use of growth patterns and other tree-ring variables. Though, we acknowledge the large remaining gaps in the understanding of mechanisms driving δ²H-fractions and how this is linked to C partitioning and/or growth. Controlled experiments are needed to establish quantitative isotope fractionation scheme. Upcoming research will benefit from the newest method developments in equilibrium methods, promising cheaper and more rapid δ²H analyses of organic material (Filot et al., 2006; Sauer et al., 2009; Wassenaar et al., 2015).

We therefore predict that the knowledge on δ²H-fractions in plants will quickly increase in the next decade. Thus, this report intends to stimulate scientists to consider δ²Hc as a potential tool in their future research and to apply the new concept and methods, which could find widespread application in modern- and paleo-tree physiological and ecological studies focusing on climate extremes, carbon starvation under drought, abiotic and biotic disturbances, and forest mortality.

Author contributions

MML, MS, and VV planned and designed the research. MML and PS performed the literature research. ML performed isotopic analyses. All authors contributed to the writing of the manuscript.

Declaration of Competing Interest

The authors declare no conflict of interest.

Acknowledgments

We acknowledge the EU project ”ISONET“ (No. EVK2-CT-2002-00147) and Hogne Junger for providing data of the Norwegian site. Our work was supported by the SNF project “IsoDrought” (No. 182092, granted to MS), by the SNF Ambizione project “TreeCarbo” (No. 179978, granted to MML), by the SNF project “Alpine Holocene tree ring isotope records” (No. 144255, granted to ML), and by the SNF Sinergia project “tTREE” (No. 136295).

References


