Evaluating climate signal recorded in tree ring $\delta^{13}C$ and $\delta^{18}O$ values from bulk wood and α-cellulose for six species across four sites in the northeastern US

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Abstract

Rationale: We evaluated the applicability of tree ring $\delta^{13}C$ and $\delta^{18}O$ values in bulk wood – instead of the more time and lab-consuming α-cellulose $\delta^{13}C$ and $\delta^{18}O$ values, for assessing climate and physiological signals across multiple sites and for six tree species along a latitudinal gradient (35°97' N to 45°20' N) of the northeastern United States.

Methods: Wood cores (*n*=4 per tree) were sampled from ten trees per species. Cores were cross-dated within and across trees at each site, and for the last 30 years. Seven years, including the driest on record, were selected for this study. The $\delta^{13}C$ and $\delta^{18}O$ values were measured on two of the ten trees from the bulk wood and the *α*-cellulose. The offsets between materials in $\delta^{13}C$ and $\delta^{18}O$ values were assessed. Correlation and multiple regression analyses were used to evaluate the strength of the climate signal across sites. Finally the relationship between $\delta^{13}C$ and $\delta^{18}O$ values in bulk wood vs α-cellulose was analyzed to assess the consistency of the interpretation, in terms of CO_2 assimilation and stomatal conductance, from both materials.

Results: We found an offset of 1.1‰ and 5.6‰ between bulk and α-cellulose for $\delta^{13}C$ and $\delta^{18}O$ values, respectively, consistent with offset values reported in the literature. Bulk wood showed similar or stronger correlations to climate parameters than α-cellulose for the investigated sites. In particular, temperature and vapour pressure deficit and SPEI were the most visible climate signals recorded in $\delta^{13}C$ and $\delta^{18}O$ values, respectively. For most of the species, there was no relationship between $\delta^{13}C$ and $\delta^{18}O$ values, regardless of the wood material considered.

Conclusions: *α*-cellulose extraction was not necessary to detect climate signals in tree rings across the four investigated sites. Furthermore, the physiological information inferred from the dual isotope approach was similar for most of the species regardless of the material considered.

Key words: tree rings, stable isotopes, bulk wood, cellulose, mesic forests

Introduction

The use of stable carbon ($\delta^{13}C$ values) and oxygen ($\delta^{18}O$ values) isotope compositions in tree rings has increased over the last two decades, contributing substantially to improving our understanding of how tree species worldwide are responding to climate changes [1, 2, 3, 4, 5, 6]. Yet despite their prevalence as climatic or ecophysiological proxies, considerable uncertainty remains regarding which wood component of tree rings is best suited for stable isotope analysis [1, 7, 8, 9, 10, 11, 12, 13].

Several studies investigated the signal coherence between bulk wood and cellulose $\delta^{13}C$ and $\delta^{18}O$ values and climate parameters, with mixed results. Some studies reported that bulk wood yielded similar or stronger relationships with climate than cellulose for $\delta^{13}C$ [14, 15, 16, 17] and $\delta^{18}O$ [18] values, or both [19, 20], while others demonstrated a diluted climate signal in bulk wood for both $\delta^{13}C$ and $\delta^{18}O$ values [21, 22, 23]. Many of these studies are limited in the number of samples, species and sites tested, thus restricting the generality of their conclusions. Exceptions to this include a global study by Barbour *et al*[18], which included $\delta^{18}O$ values, and a multi-species analysis at two sites in Southern Germany by Weigt *et a*.[20] that considered both $\delta^{13}C$ and $\delta^{18}O$ values. Both studies concluded that cellulose extraction was an unnecessary step for detecting climate signals in tree ring-isotope investigations.

Tree-ring $\delta^{13}C$ and $\delta^{18}O$ values have been extensively used to assess long-term changes in tree water-use efficiency (WUE), i.e., the ratio between CO_2 assimilation (A) and stomatal conductance (g_s), in response to climatic and anthropogenic factors (e.g., elevated CO_2, nitrogen deposition) [24, 25, 26, 27, 28, 29, 30]. This approach is based on the well-established theory for the physiology of C_3 photosynthesis, linking bulk leaf C isotope discrimination, $\Delta^{13}C$, and the ratio of the CO_2 in the intercellular spaces, c_i, and that in the atmosphere, c_a (i.e., c_i/c_a) [31]. However, many studies used cellulose $\delta^{13}C$ values as the basis for their calculations, except where efforts have been made to follow the original model [31] by correcting for the offset in

isotopic composition between bulk tissue and cellulose [4, 10, 32]. In addition to $\delta^{13}C$ values, the measure of $\delta^{18}O$ values offers insight into the role of g_s in the leaf c_i/c_a ratio and by extension, the WUE [33, 34, 35]. The $\delta^{18}O$ value in plant organic matter reflects that of the leaf water where it was formed [36], which, in turn, is affected by the $\delta^{18}O$ value of the source water [37], including meteoric, soil and atmospheric water, and isotopic fractionations occurring during transpiration, as determined primarily by g_s [12, 35, 38, 39, 40, 41, 42]. Since $\delta^{13}C$ and $\delta^{18}O$ values share their dependence on g_s, a significant relationship between the two implies that variations in the c_i/c_a ratio and WUE are affected by changes in g_s [33] (e.g., under a gradient of moisture conditions or changes in vapour pressure deficit). In contrast, variations in *A* are only reflected in changes in $\Delta^{13}C$ and hence in the $\delta^{13}C$ value, which is reflected in the absence of a relationship between the C and O isotope ratios. Whether the relationship between $\delta^{13}C$ and $\delta^{18}O$ values is consistent regardless of the plant material considered (i.e., bulk wood or cellulose) remains an unresolved question. Answering this question can be crucial to optimizing the use of the dual isotope approach [34] for advancing our understanding of tree physiological responses to climate variability.

This study aims to evaluate whether there are differences between bulk wood and *α*-cellulose in the climate and physiological information (e.g., changes in *A* and g_s) derived from the combination of $\delta^{13}C$ and $\delta^{18}O$ values across different tree species at four sites along a latitudinal gradient in the northeastern USA. Our specific goals were: 1) to document the offset between bulk wood and α-cellulose for $\delta^{13}C$ and $\delta^{18}O$ values across multiple species and sites, 2) to evaluate whether both materials reflect similar climate signals. For $\delta^{18}O$ values, we also tested whether the $\delta^{18}O$ value of source water (i.e., soil water) is recorded in both bulk wood and *α*-cellulose, and finally 3) to investigate whether the strength and directionality of the relationship between $\delta^{13}C$ and $\delta^{18}O$ values, which is often used to infer changes in *A* and g_s, remained similar between bulk wood and α-cellulose.

Materials and Methods

Study sites and sampling methods

Four forests were considered in this study, which included some of the important tree species in the northeastern USA (Table 1). Sites included mesic mature forests within two main climate zones, according to the Koppen-Geiger classification [43]: Cfa - Warm temperate climate, fully humid with hot summer (Duke Forest in North Carolina; Silas Little in New Jersey) and Dfb - Snow climate, fully humid with warm summer (Harvard Forest in Massachusetts; Howland in Maine). Detailed description of the sites can be found in Guerrieri *et al*. [44] Along the latitudinal gradient (35°97' N to 45°20' N) and over the study years (Figure 1), the mean annual temperature (T_a) ranged from 15 ^{o}C to 6 ^{o}C, while the mean annual precipitation (P_a) showed similar values (Table 1). For each site, ten trees from the two-dominant species were selected, and four wood cores were sampled from each tree. The tree species included three conifers: hemlock (*Tsuga canadensis* L. Carr.*)*, red spruce (*Picea rubens* Sarg.), shortleaf pine (*Pinus echinata* Mill.); two ring-porous broadleaved species: red oak (*Quercus rubra* L.), chestnut oak *(Quercus prinus* L.); and one semi-ring-porous species: hickory *(Carya tomentosa* L.*)* species. The *Tsuga canadensis* was a common species at two sites, Harvard Forest and Howland.

Sample preparation and stable isotope measurements

The wood cores were dated from the bark to the pith and ring width measurements were carried out with a sliding scale micrometer (Velmex, Bloomfield, NY, USA) using MeasureJ2X software (VoorTech Consulting, Holderness, NH, USA). Ring width series were crossdated first within each tree and then among trees using COFECHA [45]. At each site and for each species, the five cross-dated trees with the highest correlations with the master chronology were selected for isotopic analyses and two of them were considered for the

current study. The average age of the trees was 80-100 years. The years identified for the present study are within the last two decades. This prevent noise related to the juvenile effect and changes in the lignin:cellulose ratio at the heartwood:sapwood boundary when interpreting the isotopic signal.

Each annual ring was separated and then cut in smaller pieces by using a razor blade. For the conifer and semi-ring-porous species, the whole annual ring was separated, while in the case of deciduous species we subsampled only the late wood. We selected 7 years (Table 1, Figure 1) out of the last 30 years, which included a dry year, as well as years with no significant changes in moisture conditions. In particular, for the Harvard Forest and Howland sites, target years were identified by examining the difference between each year's growing season P_a and vapour pressure deficit, VPD, and the annual mean, calculated from available site-level climate data (1992-2006 for Harvard Forest and 1996-2004 for Howland). The year showing the highest difference from the mean VPD and mean P_a and negative values for the standard precipitation-evaporation index, SPEI (Figure S1, supporting information) was considered the 'dry year'. For the other two sites (i.e., Duke Forest and Silas Little), the dry years were identified based on the site description provided in published papers [46, 47, 48]. For each of the selected rings, 30% of the wood material was kept as a bulk, while 70% of it was used for α-cellulose extraction, according to the procedure described by [9, 49].

An amount of 0.3-0.4 mg of bulk wood and α-cellulose samples for each investigated year was weighed in tin capsules, and converted to CO_2 with an elemental analyzer (ECS 4010, Costech Analytical, Valencia, CA, USA) coupled to a continuous flow isotope ratio mass spectrometer (Delta PlusXP, ThermoFinnigan, Bremen, Germany) to determine $\delta^{13}C$ and %C values. An additional 0.3 and 0.5 mg of each sample was weighed in silver capsules, converted to CO with a pyrolysis elemental analyzer (TC/EA, ThermoFinnigan) and analyzed for $\delta^{18}O$ values with a continuous flow isotope ratio mass spectrometer (Delta PlusXP,

ThermoFinnigan). Carbon and oxygen isotope ratios were expressed in per mil (‰) relative to the V-PDB and V-SMOW international standards, respectively. All isotope analyses were carried out at the Stable Isotope Core Laboratory (School of Biological Sciences, Washington State University, Pullman, WA, USA). The standard deviations for internal standards were less than 0.2 ‰ and 0.4 ‰ for $\delta^{13}C$ and $\delta^{18}O$ values, respectively.

Modelling $\delta^{18}O$ values of source water

We estimated the $\delta^{18}O$ values in soil water ($\delta^{18}O_{sw}$), based on direct measurements of the $\delta^{18}O_{sw}$ values in the first 10-15 cm depth, sampled at monthly resolution in 2005 and 2006 at Harvard Forest (Dawson T and Munger W, personal communication), which reflects the isotopic signature of precipitation, modified by evaporation processes [18, 50]. For modelling $\delta^{18}O_{sw}$ values we used a regression analyses with P_a, T_a, and both together as independent predictors. This approach is similar to the one reported by Barbour *et al* [18], which is based on results presented by IAEA[51], except that we did not include the elevation (m *asl*) term in the analysis as it does not change across the investigated sites. The latitudinal effect on precipitation $\delta^{18}O$ values [52], which is then reflected in the $\delta^{18}O_{sw}$ values, is partially accounted for by T_a in the regression model. Selection of the best model was based on the Adjusted R^2, but also on the Akaike information criterion, AIC values. The two models containing only T_a, and both P_a, and T_a as regressors had similar R^2; however, we chose the model with T_a only, because it showed lower AIC values (Table S1, supporting information). The modelled $\delta^{18}O_{sw}$ values did not change over time at Harvard and Duke forests, while the values significantly increased at Silas Little (slope = 0.03 ‰, R^2= 0.19, p<0.05) and decreased (slope= -0.04 ‰, R^2= 0.18, p<0.05) at Howland (data not shown).

Statistical analyses

The normality and variance heterogeneity of isotopic data were assessed with the Shapiro and Levene tests, respectively. An independent sample *t*-test was performed to detect significant differences between bulk and α-cellulose for $\delta^{13}C$ and $\delta^{18}O$ values across sites and tree species. The non-parametric Kruskal-Wallis test was used when data did not conform to a normal distribution (i.e., in the case of %C in bulk wood).

Partial correlation analyses and the Pearson product-moment correlation coefficient were used to assess correlations between $\delta^{13}C$ and $\delta^{18}O$ values in both bulk and α-cellulose *vs* environmental parameters: temperature (T), vapour pressure deficit (VPD), and standardized precipitation evaporation index (SPEI) for the month of August with 3 months' lag. Correlations were performed by considering *i)* mean annual value for T and VPD (T_a, VPD_a) and *ii)* growing season mean (grs) from May to August (T_{grs} and VPD_{grs}). SPEI values were obtained from the global database available online (http://sac.csic.es/spei/).

Linear mixed effects models, LME (R package nlme)[53] with the individual trees per species as random variable (7 years as replicates each) were applied to assess the relationship between bulk wood and α-cellulose for both $\delta^{18}O$ and $\delta^{13}C$ values, to account for variation among species and sites of the model intercept. We started with the generalized linear model (GLM), and then introduced the random factors: 'Site' and 'Species nested in Site'. The model with the minimum AIC values was considered when comparing the GLM and LME models, and the LME models, in this latter case by performing an ANOVA test (Table S2, supporting information). For the best model, we also calculated the conditional and marginal coefficient of determination (package MuMIn) [54], which indicate the variance explained by both fixed and random factors and only the fixed factor, respectively.

Finally, multiple regression analyses were used to assess which of the climate variables accounted for most of the cross-site variation in tree-ring $\delta^{13}C$ and $\delta^{18}O$ values and to test

whether bulk wood or α-cellulose showed the strongest correlations with climate. Sites and tree id per species were included altogether in the analyses and we ran separate models for annual and growing season T and VPD. The variance inflation factor (VIF) was checked to ensure that all the predictors in the model had a VIF less than 4, which indicates a minimal multi-collinearity. Because of the collinearity between $\delta^{18}O_{sw}$ values and T_a/T_{grs}, we only included the isotopic composition of source water ($\delta^{18}O_{sw}$) in the model for $\delta^{18}O$ VALUES OF bulk wood and α-cellulose. We used R project statistical computing [55] for all the statistical analyses.

Results

$\delta^{13}C$, $\delta^{18}O$ and %C values measured in bulk and α-cellulose

In the following sections, the $\delta^{13}C$ and $\delta^{18}O$ values for α-cellulose will have the subscript c ($\delta^{13}C_c$ and $\delta^{18}O_c$) and the bulk wood will have the subscript b ($\delta^{13}C_b$ and $\delta^{18}O_b$). The Harvard and Howland forests are referred to as 'high latitude sites' and the Silas Little and Duke forests as 'low latitude sites'.

Differences between bulk and α-cellulose were clearer for $\delta^{18}O$ than for $\delta^{13}C$ values, which showed a higher variability between species and tree ids (Figure 1). Overall, α-cellulose showed higher $\delta^{13}C$ and $\delta^{18}O$ values than bulk wood (Table 2), by an average of 1.1‰ and 5.6‰, respectively. These results were consistent when we stratified by tree species, with the exception of *Quercus rubra* at Harvard Forest, which did not show significant differences between bulk and α-cellulose for $\delta^{13}C$ values (Table 2). For both materials, high latitude sites showed significantly less negative $\delta^{13}C$ values ($\delta^{13}C_b$= -23.4‰, $\delta^{13}C_c$ = -22.7‰) than the lower latitude sites ($\delta^{13}C_b$= -24.7‰, $\delta^{13}C_c$= -23.2‰) (Figures 2a and b). The measured $\delta^{18}O$ values for high latitude sites were lower in both bulk ($\delta^{18}O_b$ =

22.2‰) and α-cellulose ($\delta^{18}O_c$= 28.1‰) than for sites at southernmost latitude ($\delta^{18}O_b$ = 24.8‰, $\delta^{18}O_c$ = 30‰) (Figures 2c and d).

In the case of bulk wood, there was a significant difference in %C between sites at higher latitude than those at the lower latitude (Krustal-Wallis $\chi^2 = 43.77$, $p< 0.001$). The difference between bulk wood and α-cellulose for %C was higher for species at the northern than the southern latitude sites (Figure S2, supporting information), with mean values ranging from 13.0 ± 6.0 % to 5.4± 1.9 %, respectively.

We found a significant and positive relationship between bulk and α-cellulose for both carbon and oxygen isotope ratios (Figures 3a and b) and for all sites, with a slope of 0.6 and 0.8 for $\delta^{13}C$ and $\delta^{18}O$ values, respectively. However, when considering a linear-mixed effect model, the relationship between materials for both carbon and oxygen isotope ratios was improved and most of the variance was explained by the random factor, i.e., Site and Species (Table 3).

Correlations and regressions between $\delta^{13}C$ and δ values and site parameters

The $\delta^{13}C$ and $\delta^{18}O$ values measured in both bulk wood and α-cellulose showed significant correlations with climate variables (T_a, VPD_a, T_{grs}), with higher correlation coefficients obtained for bulk wood (Table 4). For conifer species, correlations with climate variables and $\delta^{13}C$ values were only significant in the bulk wood, while only α-cellulose showed significant correlations in the case of broadleaves (data not shown). Both conifer and broadleaf species had significant correlations between climate parameters and $\delta^{18}O$ values in both bulk wood and α-cellulose (data not shown). Furthermore, both materials showed significant correlations between $\delta^{18}O$ and $\delta^{18}O_{sw}$ values, with slightly higher correlation coefficients in the case of the bulk wood.

Temperature was the only parameter with a significant and negative relationship with $\delta^{13}C$ values; this trend was consistent for both bulk wood and α-cellulose (Table 5). By contrast, the bulk $\delta^{18}O$ values recorded the signal of the oxygen isotopic composition of the source water, $\delta^{18}O_{sw}$, which was lost when we considered the $\delta^{18}O$ values in α-cellulose. However, we found a significant relationship between VPD and SPEI and $\delta^{18}O$ values in both materials (Table 5).

Relationship between $\delta^{13}C$ and $\delta^{18}O$ values in bulk wood and α-cellulose

The differences across sites for the $\delta^{18}O$ values of source water or atmospheric water vapour may affect the interpretation of the $\delta^{18}O$ values measured in tree rings, when all species (and sites) are considered together. We therefore explored relationships between the stable carbon and oxygen isotopic compositions at the species level within each site (Figure 4). When considering the bulk wood, we did not find a significant relationship between $\delta^{13}C$ and $\delta^{18}O$ values for any of the investigated species. Similarly, for α-cellulose, most of the species did not show a significant relationship between the C and O isotope ratios, with two notable exceptions: *Quercus rubra* at Harvard Forest (R^2= 0.67, slope=1.61, p<0.001) and *Tsuga canadensis* at Howland (R^2=0.53, slope= 1.10, p<0.01).

Discussion

Offset between bulk wood and α-cellulose for $\delta^{13}C$ and $\delta^{18}O$ and %C values

Significant differences were found in the isotopic compositions of bulk wood and *α*-cellulose, with higher $\delta^{13}C$ and $\delta^{18}O$ values for *α*-cellulose. The offset of 1.1‰ and 5.6‰ between bulk and *α*-cellulose for C and O isotope ratios, respectively, is within the range of values reported in previous studies for $\delta^{13}C$ [13, 14, 15, 16, 32, 56, 57, 58, 59, 60, 61, 62] and $\delta^{18}O$ values [16, 18, 62, 63].

Extracted α-cellulose isolated from different tree species had a relative %C within the reported range of 41-45% from the literature and it is close to the theoretical value of 44.45% [64]. This suggests that the α-cellulose quality was high. Interestingly, the difference between bulk wood and α-cellulose for %C was higher in the case of species at the northern sites than those at the more southern sites. This could be attributed to a higher proportion of extractives being removed at the northern sites, with more conifer species than the other two sites (Table 1). However, Harlow *et al*[10] reported higher %C for the extractive free wood than for bulk wood for over 40 species in the USA, which included both coniferous and deciduous species. The observed differences between sites at different latitudes for %C in bulk wood could indicate that species at the northern sites have a higher lignin:cellulose ratio than those at the southern sites. Indeed, lignin content is the greatest source of the difference in $\delta^{13}C$ values between total wood and cellulose [58]. Higher lignin content, which implies a higher C content [65], was found for softwoods than for hardwoods [66]. Our results for bulk wood agree with previous studies, where significant differences among species for C content in wood were observed [66, 67].

Comparing bulk and α-cellulose for the climate signal detected from tree-ring $\delta^{13}C$ and $\delta^{18}O$ values

Tree-ring cellulose has often been considered as the best material for assessing the climatic signal recorded in tree ring C and O isotope ratios because it is a relatively pure, with consistent structure, known biosynthetic pathway, and low mobility between annual rings, so that its isotopic composition and interpretation are considered more predictable than those of bulk wood [1,3,12,68]. Overall, we found that across the multiple species at the four sites, climate signals were similarly or more strongly recorded in bulk wood than in α-cellulose, particularly for $\delta^{13}C$ values (Tables 4 and 5). This suggests that cellulose extraction may be

unnecessary when the goal is to capture a regional scale climate signal on a short-time window. This agrees with previous studies, which found that bulk wood provides the same climate signal as cellulose for $\delta^{13}C$ values [14, 16, 20, 69]. More contrasting results have been reported for $\delta^{18}O$ values as to whether bulk wood [18, 63, 70] or cellulose [14, 16, 41, 71] shows stronger or similar [72] correlations with climate.

The combination of tree-ring $\delta^{13}C$ and $\delta^{18}O$ values provided complementary climate information. Tree-ring $\delta^{13}C$ values were sensitive to T, while $\delta^{18}O$ values to VPD and SPEI, consistent with previous studies [3,6,16, 20, 70]. The positive relationship between SPEI and $\delta^{18}O$ values observed in this study is contrary to that expected, as lower SPEI is typically associated with greater moisture stress and, consequently, a reduction in leaf transpiration. This would presumably translate in higher leaf water ^{18}O enrichment due to a lower mix between unenriched water from the xylem (which has the same isotopic composition as the soil water) and ^{18}O-enriched water at the leaf evaporative sites [38], which is then partially reflected in the $\delta^{18}O$ values measured in tree rings [36]. However, these assumptions may not apply to mesic forests occurring along a latitudinal gradient mostly driven by changes in T, rather than by moisture conditions. The relationship between $\delta^{18}O$ values and climate suggests that VPD might constrain transpiration more than soil moisture for mesic forests [73].

The stronger relationship between $\delta^{13}C$ and $\delta^{18}O$ values and site-parameters in the case of the bulk wood could be partially explained by the difference across species along the investigated gradient in the lignin:cellulose ratio [8] and its link to xylem cell development during radial growth. Tree-ring formation consists of two main cellular processes: new xylem cell production and enlargement (radial growth) and deposition of cellulose, hemicellulose and at last lignin, to build the secondary walls (cell wall thickening) [74, 75]. Lignin deposition is a high C demand process extending beyond tree radial growth [76] and cellulose deposition, and it has been shown to be highly sensitive to temperature [74,77,78,79]. Preserving lignin may

increase the robustness of the temperature signal, the detection of a temperature gradient and its influence on physiological processes (e.g., investment of current year photosynthates in lignin deposition) toward the late growing season, if the bulk wood $\delta^{13}C$ value is considered when investigating species across regions. Similar patterns for $\delta^{18}O$ values were observed in the meta-analysis by Barbour *et al*.[18] However, the relationship between whole wood (including lignin) and cellulose is subject to change over long time periods (e.g. from changes in climate, CO_2 concentration, wood composition), such that reconstructing climate from tree rings spanning centuries in paleoclimatic studies needs to be done cautiously [17].

Finally, another advantage of using bulk wood identified in our study is that it carries a stronger fingerprint of the $\delta^{18}O_{sw}$ values than α-cellulose (Tables 4 and 5) for tree-ring $\delta^{18}O$ values. This finding indicates that the isotopic signature of the source water is an important predictor of tree-ring $\delta^{18}O$ values in the absence of soil water limitation. Barbour *et al*[18] showed that for *Quercus* and *Pinus* ssp., the $\delta^{18}O$ values in lignin reflected not only the isotopic signal of the molecular oxygen during lignin synthesis, but also the leaf and source water $\delta^{18}O$ values.

Do bulk wood and α-cellulose carry similar physiological information to that derived from the dual isotope approach?

While the most common use of the dual isotope approach [34] is to look at different combinations of shifts in $\delta^{13}C$ *vs* $\delta^{18}O$ values to assess changes of A and g_s among species in response to climate [80,81,82,83,84] or to treatments [6,30,85], a number of studies looked at the relationship between $\delta^{13}C$ and $\delta^{18}O$ values measured in tree rings [33,80,81].

In this study, we were particularly interested in assessing whether the directionality and strength of the relationship between $\delta^{13}C$ and $\delta^{18}O$ values were similar, regardless of the material considered. For the two species at Silas Little and Duke Forest, and one species at

Harvard Forest (*Tsuga canadensis*) and Howland (*Picea rubens*), no significant relationship between $\delta^{13}C$ and $\delta^{18}O$ values was observed for both materials (Figure 4), suggesting that interpretation of the results, in terms of changes in *A* and/or g_s, would not be affected by the material used for analyses. Conversely, for *Quercus rubra* at Harvard Forest and *Tsuga canadensis* at Howland, significant and positive relationships were observed between $\delta^{13}C$ and $\delta^{18}O$ values for α-cellulose but not for bulk wood. In this case, the interpretation of the major controls on changes in c_i/c_a and WUE based on the dual isotope approach would differ depending on the used plant material. This diverging result could be partially related to the seasonal changes in $\delta^{18}O$ values in precipitation and differences in water depth accessed by trees, which both will determine the isotopic signature of soil water, and by extension the tree-ring $\delta^{18}O$ values [86]. At Howland, we did find a significant change over the investigated years in the modelled $\delta^{18}O_{sw}$ values, which might help to explain the difference between the two materials for the relationship between $\delta^{13}C$ and $\delta^{18}O$ values. However, this was not the case for $\delta^{18}O_{sw}$ values at Harvard Forest, which did not show significant changes over time.

We acknowledge that the dual isotope approach should be used with caution, especially when interpreting qualitative changes in the integrated g_S from tree-ring $\delta^{18}O$ values [87]. However, this approach could be improved by using site-specific conditions [87] and also tree-specific physiological and functional traits [44, 87].

Conclusion

Our study offers useful insight for the future directions of dendroisotopic research seeking to understand SPATIAL AND TEMPORAL PATTERNS OF TREE CARBON AND WATER USE STRATEGIES under climate change across regional networks of multiple sites and species. We found that extraction of α-cellulose can be avoided when aiming at detecting the climate

signal across the four investigated sites and species along a latitudinal gradient of mesic forests northeastern USA. Our results further suggest that removing lignin may reduce the detection of the climate signal toward the end of the growing season for both $\delta^{13}C$ and $\delta^{18}O$ values and for the $\delta^{18}O$ values of source water in the case of tree-ring $\delta^{18}O$ values. Moreover, considering both $\delta^{13}C$ and $\delta^{18}O$ values allows complementary climate information to be gained, with T alone and VPD and SPEI combined, serving as the best predictors for $\delta^{13}C$ and $\delta^{18}O$ values, respectively. Therefore, measuring both C and O isotope ratios contributes to improving the interpretation of plant physiology-climate interactions. Finally, α-cellulose extraction can be reliably avoided for sites where the physiological information using the dual isotope approach was similar for all species regardless of the material considered.

We acknowledge that our results rely on a limited number of trees and might require confirmation when longer time series are considered. Nevertheless, they suggest that it could be good practice to conduct a site- and species-specific preliminary analysis to determine whether α-cellulose extraction is required before relying solely on bulk wood for dendroisotopic studies.

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Table 1 Forest sites, tree species and years considered in the study. The dry years are indicated in bold. T_a, P_a, VPD_a indicate the annual mean of temperature, precipitation and vapor pressure deficit, respectively, while T_{grs}, P_{grs} and VPD_{grs} the mean values of temperature, precipitation and vapor pressure deficit over the growing season, grs (May-August), calculated for the years considered in this study. Lat and Long indicate Latitude and Longitude, respectively.

Site	Lat °N	Long °W	Sampled species	Years included in this study	T_a (°C)	P_a (mm)	VPD_a (kPa)	T_{grs} (°C)	P_{grs} (mm)	VPD_{grs} (kPa)
Duke Forest	35°97'	79°10'	*Carya tomentosa* L. (hickory)	2000,2001,**2002**,2003,2004,**2005**,2006	15.13	1036	0.57	22.24	487	0.68
Harvard Forest	42°54'	72°17'	*Quercus rubra* L. (red oak)	1997,1998,**1999**,2000,2001,2002,2003	7.85	1144	0.35	18.92	359	0.53
			Tsuga canadensis L. Carr. (hemlock)							
Howland	45°20'	68°74'	*Picea rubens* Sarg. (red spruce)	1996,**1997**,1998,1999, 2003,2004,2005	6.64	858	0.45	16.65	443	0.68
			Tsuga canadensis L. Carr. (hemlock)							
Silas Little	39°91'	74°60'	*Quercus prinus* L. (chestnut oak)	2004,**2005**, 2006,2007, **2010**,2011,2012	12.71	1123	0.53	21.49	476	0.78
			Pinus echinata Mill. (shortleaf pine)	2003, 2004,**2005**, 2006,2009,**2010**,2011						

Table 2. Results from the paired sample *t*-test assessing differences between bulk and α-cellulose for the $\delta^{13}C$ and $\delta^{18}O$ values measured for the years reported in Figure 1 and Table 1. The analyses were carried out by i) considering all species together (All Species), and ii) keeping separated each of the tree species included in the study. (*), (**) and (***) indicate $p< 0.05$, $p< 0.01$ and $p< 0.001$, respectively.

Site	**Species**	**$\delta^{13}C$ (‰)**				**$\delta^{18}O$ (‰)**			
		Bulk wood	*α*-cellulose	*t*-value	*p*	bulk	*α*-cellulose	*t*-value	*p*
All sites	All species	-23.9	-22.9	-5.97	***	25.2	29.0	-20.31	***
Duke Forest	*Carya tomentosa*	-24.5	-23.3	-4.64	***	24.1	28.4	-11.34	***
Harvard	*Quercus rubra*	-24.2	-24.1	-0.42	*n.s.*	25.2	29.0	-12.92	***
Forest	*Tsuga canadensis*	-22.7	-21.8	-3.30	**	21.9	28.1	-13.21	***
Howland	*Tsuga canadensis*	-24.5	-23.0	-5.38	***	22.7	29.2	-17.30	***
	Picea rubens	-22.3	-21.4	-2.53	*	22.4	28.0	-15.78	***
Silas Little	*Quercus prinus*	-25.0	-23.7	-4.13	***	25.2	30.1	-11.54	***
	Pinus echinata	-24.5	-22.6	-4.05	***	25.2	31.4	-21.23	***

Table 3. Descriptive statistic of the Linear mixed effects model. Fixed factor is the $\delta^{13}C$ and $\delta^{18}O$ values measured in α-cellulose, while the random factor is Species nested in the Site (i.e., 1|Site/Species) for $\delta^{13}C$ values, and Site only (i.e., 1|Site) for $\delta^{18}O$ values. The selection of the model was carried out as described main text and based on results shown in the Table S2 (supporting information). Marginal R^2 and conditional R^2 indicate the VARIANCE EXPLAINED BY FIXED FACTORS ONLY AND BY BOTH RANDOM AND FIXED FACTORS, RESPECTIVELY. *** indicates $p < 0.001$

Equation	**Fixed factor**				**Marginal R^2**	**Conditional R^2**
	Estimate (β)	Standard Error	*t*-value	*p*		
$\delta^{13}C_b = \alpha + \beta \times \delta^{13}C_c + (1\|Site/Species) + \varepsilon$	0.41	0.09	4.46	***	0.19	0.56
$\delta^{18}O_b = \alpha + \beta \times \delta^{18}O_c + (1\|Site) + \varepsilon$	0.37	0.06	5.58	***	0.22	0.69

Table 4. Results from the Partial correlation analyses to explore correlation between $\delta^{13}C$ and $\delta^{18}O$ values in bulk and α-cellulose and environmental parameters: mean annual and growing season temperature (T_a and T_{grs}, respectively), mean annual and growing season vapor pressure deficit (VPD_a and VPD_{grs}, respectively), SPEI for August with 3 months' lag, modeled soil water $\delta^{18}O$ values ($\delta^{18}O_{sw}$) as described in the main text. Analyses were performed by considering all species and sites together. Pearson coefficients are reported and (*),(**) and (***) indicate $p< 0.05$, $p< 0.01$ and $p< 0.001$, respectively.

Isotopic composition	**Material**	**Parameters**					
		SPEI	**T_a**	**VPD_a**	**T_{grs}**	**VPD_{grs}**	**$\delta^{18}O_{sw}$**
$\delta^{13}C$	Bulk	-0.05	-0.43 (***)	-0.34 (**)	-0.38 (***)	-0.20	
	α-cell	-0.10	-0.23 (*)	-0.05	-0.23 (*)	0.08	
$\delta^{18}O$	Bulk	-0.17	0.70 (***)	0.73 (***)	0.61 (***)	0.64	0.70 (***)
	α-cell	-0.13	0.35 (**)	0.55 (***)	0.29 (**)	0.66 (***)	0.35 (**)

Table 5. Results from the multiple regression analyses to assess the relationship between $\delta^{13}C$ and $\delta^{18}O$ values measured in bulk wood ($\delta^{13}C_b$, $\delta^{18}O_b$) and α-cellulose ($\delta^{13}C_c$, $\delta^{18}O_c$) for all sites and environmental parameters to test for regional climate patterns along the site latitudinal gradient. Climate parameters included mean annual and growing season temperature (T_a and T_{grs}, respectively), mean annual and growing season vapor pressure deficit (VPD_a and VPD_{grs}, respectively), SPEI for August with 3 months' lag, modeled soil water $\delta^{18}O$ values ($\delta^{18}O_{sw}$) as described in the main text. Because of the collinearity between T_a/T_{grs} and $\delta^{18}O_{sw}$ values, we included only the latter in the linear models for $\delta^{18}O$ values. We report only the adjusted R^2 and the coefficient, β, when *t*-test showed β values were significantly different from zero. (*), (**) and (***) indicate $p< 0.05$, $p< 0.01$ and $p< 0.001$, respectively.

Model	R^2	β		*t*-value	*p*
$\delta^{13}C_b \sim T_a + VPD_a + SPEI$	0.23	T_a	-0.15	-2.74	**
$\delta^{13}C_b \sim T_{grs} + VPD_{grs} + SPEI$	0.18	T_{grs}	-0.23	-3.66	***
$\delta^{13}C_c \sim T_a + VPD_a + SPEI$	0.09	T_a	-0.18	-3.01	**
$\delta^{13}C_c \sim T_{grs} + VPD_{grs} + SPEI$	0.08	T_{grs}	-0.19	-3.003	**
$\delta^{18}O_b \sim VPD_a + \delta^{18}O_{sw} + SPEI$	0.58	VPD_a $\delta^{18}O_{sw}$	8.96 0.29	4.48 2.48	*** *
$\delta^{18}O_b \sim VPD_{grs} + \delta^{18}O_{sw} + SPEI$	0.66	VPD_{grs} SPEI $\delta^{18}O_{sw}$	5.94 0.32 0.51	6.55 2.14 6.86	*** * **
$\delta^{18}O_c \sim VPD_a + \delta^{18}O_{sw} + SPEI$	0.35	VPD_a	13.005	5.08	***
$\delta^{18}O_c \sim VPD_{grs} + \delta^{18}O_{sw} + SPEI$	0.50	VPD_{grs} SPEI	8.44 0.42	7.35 2.23	*** *

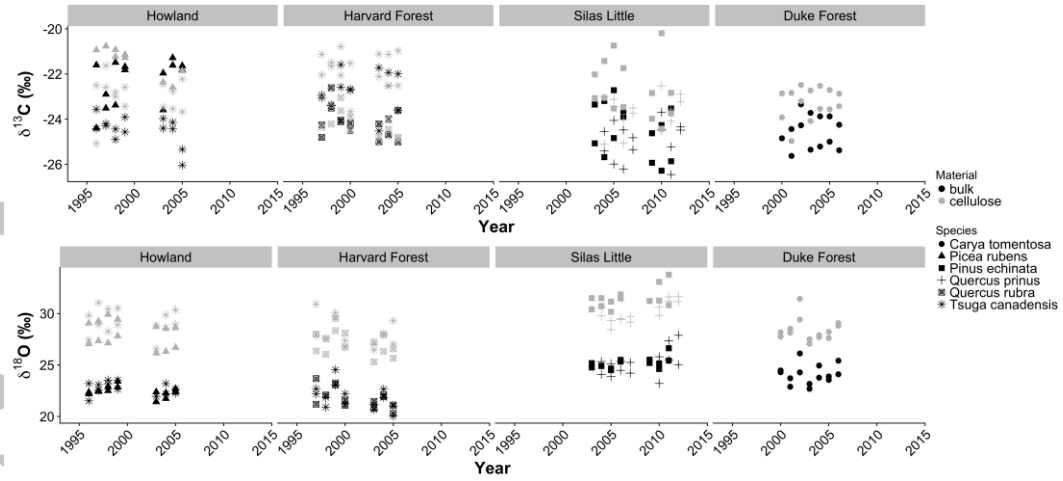

Figure 1 $\delta^{13}C$ and $\delta^{18}O$ values measured for each species (n=2 trees per species) across the seven selected years of study. Sites in the panels are arranged according to their latitude (from 45° 20′ to 35° 97′N). The dry years for each site: 1999 for Howland, 1997 for Harvard Forest, 2005 and 2010 for Silas Little and 2002 and 2005 for Duke Forest.

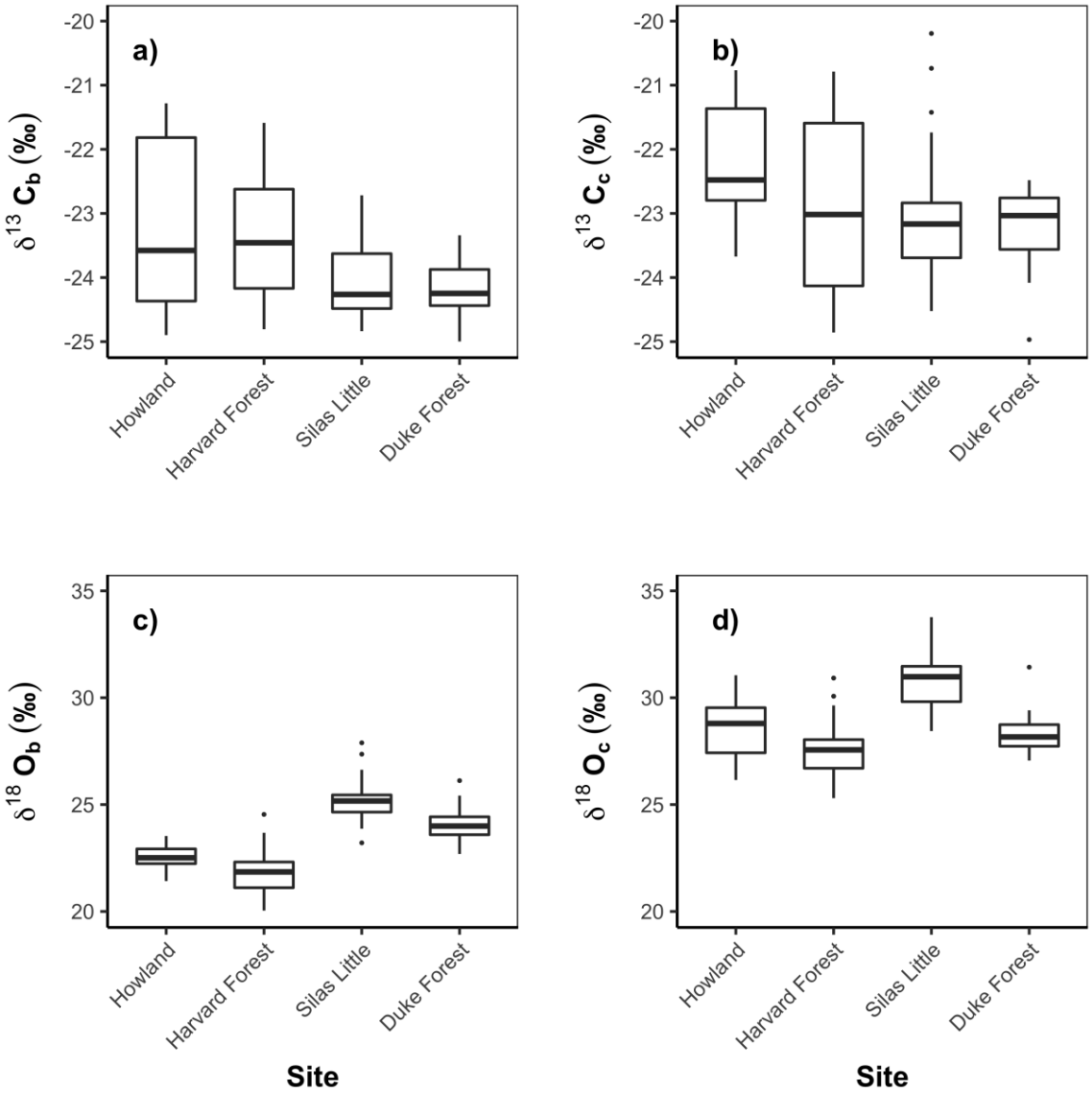

Figure 2. Boxplots showing $\delta^{13}C$ and $\delta^{18}O$ values measured in bulk ($\delta^{13}C_b$ and $\delta^{18}O_b$) and α-cellulose ($\delta^{13}C_c$ and $\delta^{18}O_c$) for the two dominant species at the four investigated AmeriFlux sites. Sites in the *x*-axis are arranged according to their latitude (from 45° 20′ to 35° 97′N).

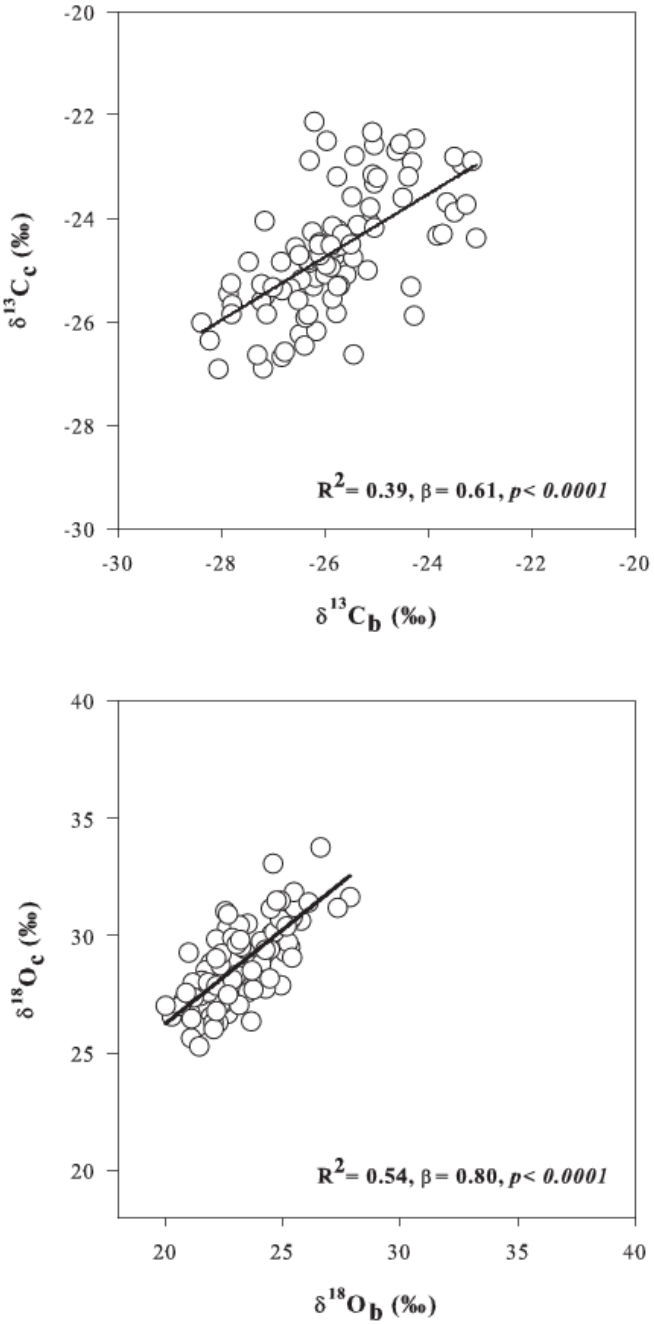

Figure 3. Relationship between bulk wood and α-cellulose for a) $\delta^{13}C$ and b) $\delta^{18}O$ values.

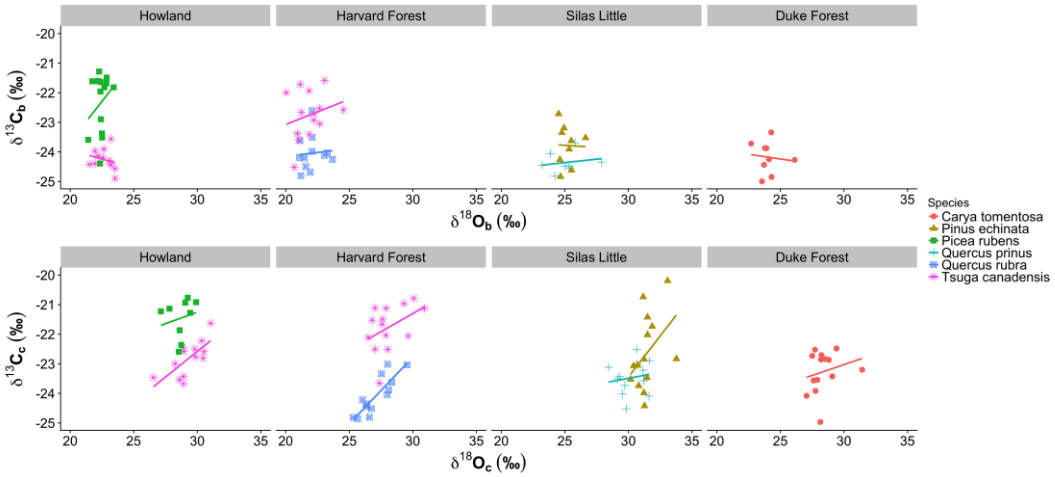

Figure 4. Relationship between $\delta^{13}C$ and $\delta^{18}O$ values measured in bulk ($\delta^{13}C_b$ and $\delta^{18}O_b$) and α-cellulose ($\delta^{13}C_c$ and $\delta^{18}O_c$) for tree species considered at each site. Relationship was significant only in the case of *Quercus rubra* at Harvard Forest (slope= 1.61, R^2= 0.67, p<0.01) and *Tsuga canadensis* at Howland (slope=1.10, R^2=0.53, p<0.01), and when α-cellulose was considered.