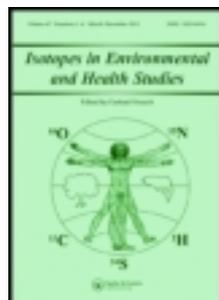


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There is no Temperature Dependence of Net Biochemical Fractionation of Hydrogen and Oxygen Isotopes in Tree-Ring Cellulose

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THERE IS NO TEMPERATURE DEPENDENCE OF NET BIOCHEMICAL FRACTIONATION OF HYDROGEN AND OXYGEN ISOTOPES IN TREE-RING CELLULOSE

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The isotopic composition of tree-ring cellulose was obtained over a two-year period from small diameter, riparian zone trees along an elevational transect in Big Cottonwood Canyon, Utah, USA to test for a possible temperature dependence of net biological fractionation during cellulose synthesis. The isotope ratios of stream water varied by only 3.6‰ and 0.2‰ in δD and $\delta^{18}O$, respectively, over an elevation change of 810 m. The similarity in stream water and macroenvironment over the short (13 km) transect produced nearly constant stem and leaf water δD and $\delta^{18}O$ values. In addition, what few seasonal variations observed in the isotopic composition of source water and atmospheric water vapor or in leaf water evaporative enrichment were experienced equally by all sites along the elevational transect. The temperature at each site along the transect spanned a range of $\geq 5^\circ C$ as calculated using the adiabatic lapse rate. Since the δD and $\delta^{18}O$ values of stem and leaf water varied little for these trees over this elevation/temperature transect, any differences in tree-ring cellulose δD and $\delta^{18}O$ values should have been associated with temperature effects on net biological fractionation. However, the slopes of the regressions of elevation *versus* the δD and $\delta^{18}O$ values of tree-ring cellulose were not significantly different from zero indicating little or no temperature dependence of net biological fractionation. Therefore, cross-site climatic reconstruction studies using the isotope ratios of cellulose need not be concerned that temperatures during the growing season have influenced results.

Keywords: Oxygen 18; Hydrogen 2; Natural variations; Tree ring cellulose; Temperature dependence; Biological fractionation

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INTRODUCTION

The hydrogen and oxygen stable isotope ratios in tree-ring cellulose have been used to quantify past climatic variation [1]. The δD and $\delta^{18}\text{O}$ values in tree-ring cellulose are derived from water and reflect, to a first approximation, precipitation inputs [2–4]. Since the isotopic composition of precipitation is a direct function of condensation temperature [5], the isotopic composition of tree-ring cellulose has been used for temperature reconstruction [6–9]. Climatic reconstructions have relied on correlations between the δD and $\delta^{18}\text{O}$ values in tree-ring cellulose and known temperature variation [7, 8, 10, 11]. However, the slope of the regression for the isotopic composition of tree-ring cellulose and precipitation is often less than 1.0, implying that other environmental factors may play a role in determining the isotope ratios in cellulose. Unless there is complete isotopic exchange with xylem water at the time of cellulose synthesis [12, 13], tree-rings are not likely to be a direct recorder of the isotopic composition of precipitation, since there are many steps along the path from source water to cellulose [14]. This uncertainty regarding what information, both biological and environmental, is contained in the δD and $\delta^{18}\text{O}$ values in tree-ring cellulose [15, 16] has provided impetus for the development of mechanistic models that mathematically describe how metabolic processes modify the isotopic composition of organic matter [17–20].

The Roden *et al.* [20] model has two major components: (a) a leaf water model that uses environmental inputs to predict the extent of evaporative enrichment for a given isotopic input of source water and atmospheric vapor and (b) a biochemical model that predicts cellulose isotopic composition based on autotrophic and heterotrophic fractionation factors as well as the extent of isotopic exchange between substrates and medium water at the site of cellulose synthesis. This model has been shown to adequately account for the various results in the literature [20] as well as those obtained from a long-term experimental system where tree rings were produced under controlled environmental conditions [14]. This model has also addressed some of the lingering questions regarding isotopic exchange with medium water [12, 13] as well as humidity signals in tree-ring cellulose [21, 22].

However, questions still remain regarding the temperature dependence of net biochemical fractionation.

The isotopic composition of tree-ring cellulose is temperature dependent since the δD and $\delta^{18}O$ values of meteoric water, which the tree takes up, are temperature dependent. However, if the biochemical fractionation events during cellulose synthesis are also temperature dependent, the interpretation of tree-ring isotopic signatures becomes more complicated. Early studies [2, 23] that addressed the temperature dependence of biochemical fractionation utilized aquatic plants and reached inconsistent conclusions (anywhere from $+4$ to $-5\text{‰}^{\circ}\text{C}^{-1}$ for δD , but no effect on $\delta^{18}O$). White *et al.* [4] studied isotope variation within the annual growth ring of white pine (a more appropriate experimental system) and suggested that the net biological fractionation factor for δD was temperature dependent with a value of $+1.3\text{‰}^{\circ}\text{C}^{-1}$. Unfortunately, not all environmental parameters necessary to determine the isotope ratio of leaf water were measured in their study, leaving this issue unresolved. Therefore, the objective of this study is to test for a possible temperature dependence of net biological fractionation during cellulose synthesis in tree rings.

METHODS AND MATERIALS

Plant Material and Field Sites

Cottonwood trees (*Populus angustifolia* James) were located in six sites along an 810 m elevational transect (from 1433 to 2243 m elevation) in Big Cottonwood Canyon near Salt Lake City, Utah, USA (from $40^{\circ}37'N$ $111^{\circ}48'W$ to $40^{\circ}38'N$ $111^{\circ}40'W$). Two to three stream side trees were tagged at each site along Big Cottonwood Creek. All trees were less than 10-cm diameter to ensure that the trees were still using the stream water rather than deeper sources [24]. The same trees were followed over two growing seasons (1996 and 1997) except when a tree died and then a replacement tree was selected. These sites were chosen for the large elevation change over a relatively short distance (13 km) and the availability of a single riparian species over the entire elevational transect and its proximity to Salt Lake City.

Isotope Sampling and Environmental Measurements

At monthly intervals approximately 5 ml of stream water was sampled at each site. During the growing season, in addition to stream water, monthly samples of twigs and leaves were collected for water extraction. Leaf material, with the mid-vein removed, and approximately 5 to 10 cm of suberized twig material were placed into separate glass vials, sealed with parafilm and brought back to the laboratory on dry ice then placed into a freezer (-5°C) until the water could be extracted for isotopic analysis. At the time of leaf and stem water collection, the ambient relative humidity and air temperature were measured and the atmospheric water vapor was sampled using a pump to draw air through a glass trap submerged in a mixture of ethanol and dry ice (-78°C) at the mid-elevation site only (1987 m). Due to the difficulty of environmental sampling at distant sites at the same time, we chose to measure temperature and humidity at one site only and calculate the ambient temperature at distant sites from the adiabatic lapse rate ($0.006^{\circ}\text{C m}^{-1}$ was used based on the average humidity at this site) [25] and assume a constant ambient vapor pressure over the entire transect (as has been shown to occur in a nearby canyon) [26]. At the end of each growing season, cores from the main stems were taken from each cardinal direction. The annual growth-ring produced during that year was cut out, dried and ground to pass a 40 mesh screen using a mill.

Sample Preparation and Analysis

Leaf water was obtained by cryogenic extraction as described by Ehleringer and Osmond [27]. The sample was frozen in liquid nitrogen and once evacuated, the system was then isolated from the vacuum pump and immersed in boiling water. The water from the leaf was then collected in a tube immersed in liquid nitrogen until all water was extracted. The δD of water samples from the stream, stems, leaves and atmospheric vapor were obtained by reducing the H in $2\ \mu\text{L}$ of H_2O to H_2 using 100 mg of a Zn catalyst (from Indiana University) in a 500°C oven (modification of Coleman [28]). The $\delta^{18}\text{O}$ of water samples were obtained by equilibrating 0.5 ml of water with approximately 16 KPa of CO_2 in a 25°C water bath for 48 h [29]. The CO_2 was extracted cryogenically using liquid nitrogen and dry-ice/ethanol traps. Both the H_2 and CO_2 were analyzed on a Finnigan MAT delta S isotope ratio

mass spectrometer (San Jose, CA) with a precision of $\pm 1\%$ for δD and $\pm 0.2\%$ for $\delta^{18}O$.

The α -cellulose of individual tree-rings was extracted for $\delta^{18}O$ measurements. We used the procedure by Leavitt and Danzer [30] which involves delipification using toluene and ethanol, boiling in water to remove soluble sugars, bleaching with sodium chlorite and acetic acid to remove lignin and proteins and washing in a strong alkaline solution to remove hemicellulose. Approximately 1.2 mg of α -cellulose was then placed in a silver capsule and converted to CO by pyrolysis [31] in a hot ($1100^\circ C$) high-purity alumina combustion column (Carla-Erba interface) and separated from other gases along a 1 m mole sieve GC column connected to a Finnigan MAT delta S isotope ratio mass spectrometer. Repeated sampling was utilized to reduce any possible memory effects and resulted in a $\delta^{18}O$ precision of $\pm 0.4\%$.

To obtain the δD recorded in tree-ring cellulose the α -cellulose was nitrated to remove the exchangeable hydrogens [31]. The α -cellulose was placed in a flask with a solution of nitric acid and acetic anhydride. The sample material was then washed, dissolved in acetone (to obtain purified tri-nitrated cellulose) and freeze dried for storage. Approximately 11 mg of nitrated cellulose was placed in a pyrex tube with 1 g of cupric oxide, evacuated, sealed and combusted for 3 h at $520^\circ C$. The resulting gases were separated cryogenically using liquid nitrogen and dry-ice/ethanol traps to move the water vapor to a tube containing the Zn catalyst for hydrogen reduction as described above.

Throughout this paper we use the conventional "delta" notation which expresses the isotopic composition of a material relative to that of a standard on a per mil deviation basis:

$$\delta = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \cdot 1000 \quad (1)$$

where δ is referred to as the isotope ratio (δD for hydrogen and $\delta^{18}O$ for oxygen). The standard for both hydrogen and oxygen is Standard Mean Ocean Water (SMOW).

RESULTS

The isotopic composition of stream water did not significantly vary over the elevational transect (Figs. 1 and 2). Although the linear

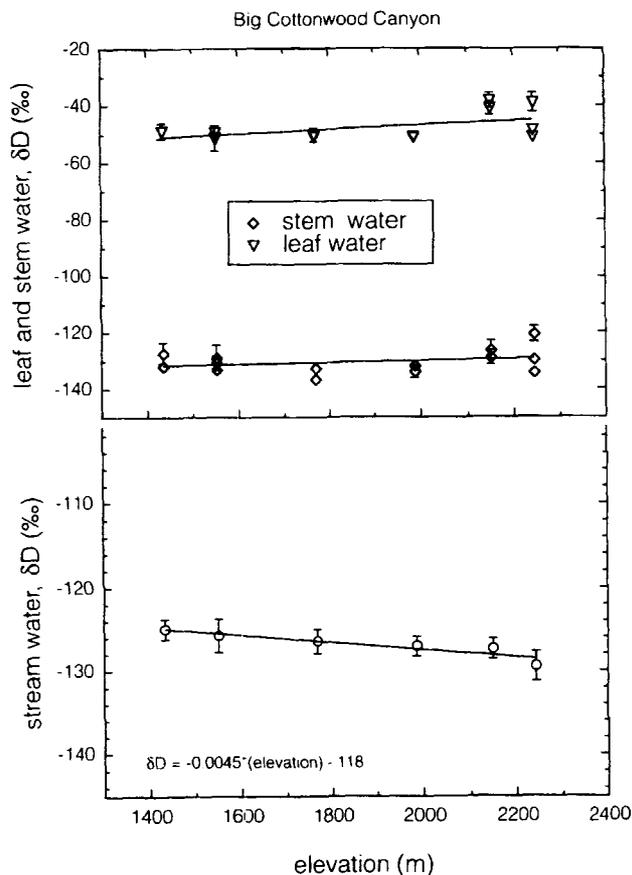


FIGURE 1 The hydrogen isotopic composition of stream, stem and leaf water across the elevational transect during the growing season over a 2 year period. Values are means and standard errors of seasonal variation. The linear equation for stream water is $\delta D_{\text{stream}} = -0.0045 \cdot (\text{elevation}) - 118$.

regression for stream water as a function of elevation indicated an isotopic enrichment across the transect of 3.6 and 0.2‰ for δD and $\delta^{18}O$ respectively, the regression (using the raw data rather than the means) was not statistically significant ($p > 0.5$). The elevational trends in the isotopic composition of stream water, although small, are expected since lower elevation sites would have input from tributaries that are less influenced from the isotopically depleted snowpack. Each data point is the mean of all growing season sampling dates. Plots of

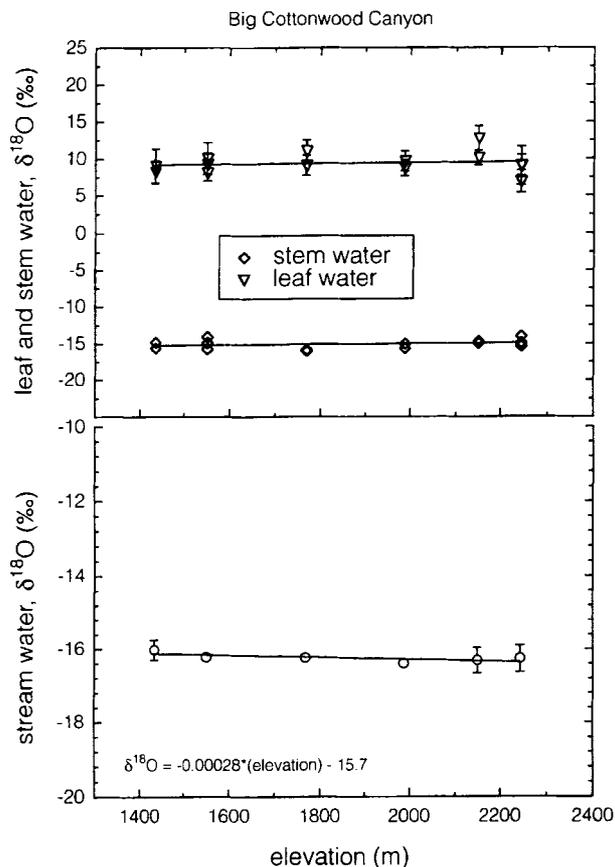


FIGURE 2 The oxygen isotopic composition of stream, stem and leaf water across the elevational transect during the growing season over a 2 year period. Values are means and standard errors of seasonal variation. The equation for stream water is $\delta^{18}\text{O}_{\text{stream}} = -0.00028 * (\text{elevation}) - 15.7$.

individual sampling dates also do not show a strong trend in isotopic enrichment across the transect (data not shown). In addition, there were little or no elevational trends in the δD and $\delta^{18}\text{O}$ of either xylem sap or leaf water (Figs. 1 and 2). The similarity between the isotopic composition of xylem sap and stream water indicated that these trees were utilizing stream water and not any possible deeper water sources. Thus, all plants along this elevational/temperature transect had similar water sources and leaf water evaporative enrichment, producing an appropriate system to test for temperature effects.

To examine seasonal variation in stream water isotopic composition, the data from each site were pooled for each sampling date (Fig. 3). In general, the δD and $\delta^{18}O$ of stream water remained relatively constant over the entire sampling period. Although the stream water varied seasonally by approximately 5 and 1‰ for δD and $\delta^{18}O$, respectively, this was well below the variation in precipitation input for this region which can vary by as much as 150‰ in δD [33]. The greatest seasonal variation in stream water isotopic composition was associated with

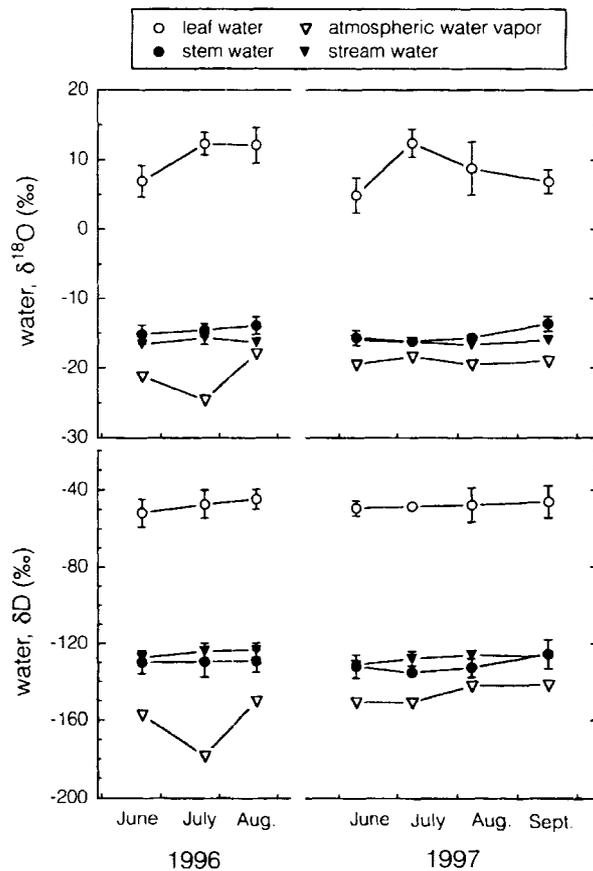


FIGURE 3 Seasonal variation in the hydrogen and oxygen isotopic composition of stream, stem and leaf water along with atmospheric water vapor for a 2 year period. Values are means and standard errors over all sites ($n=6$ for stream water, $n=1$ for atmospheric water vapor, $n=12$ for stem and leaf water).

spring snowmelt which added more isotopically depleted water into the stream. However, these small changes in stream water δD and $\delta^{18}O$ are experienced at all sites along the transect, and therefore, the trees growing at different elevations did not have significant seasonal differences in source water input. The near identical δD and $\delta^{18}O$ values of stem water and stream water indicated that these trees were indeed using stream water as their primary water source and did not change their water source throughout the growing season (Fig. 3). There was greater seasonal variation in leaf water $\delta^{18}O$ than for δD values which is associated with evaporative enrichment affecting oxygen to a greater extent than hydrogen [34]. The isotopic composition of atmospheric water vapor varied seasonally by 28 and 7‰ for δD and $\delta^{18}O$, respectively (Fig. 3).

A plot of δD versus $\delta^{18}O$ for both stem and leaf water along with the meteoric water line (Fig. 4) describes the degree of leaf evaporative enrichment experienced for each site. The stem water values fall close to the local meteoric water line [33] with a slight offset. The

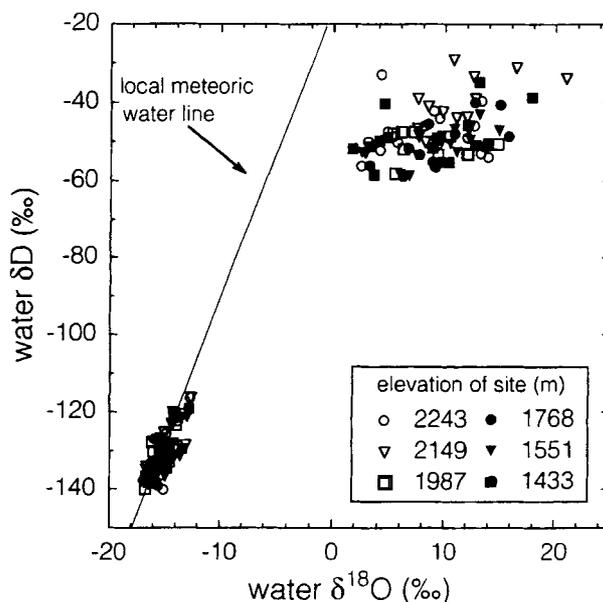


FIGURE 4 The relationship between growing season stem and leaf water $\delta^{18}O$ and δD at six different elevations.

variability in the isotopic composition of leaf water was associated with seasonal variation in humidity, temperature and the isotopic composition of atmospheric water vapor (see leaf water models [34]). The filled and open symbols in Figure 4 are the lower and higher elevation sites, respectively, and the overlap of the symbols indicate a lack of elevational trends for both leaf and source water. The lines connecting the mean source water to the mean leaf water for each site all had similar slopes (from 3.24 to 3.55), again indicating that this elevational/temperature transect produced plants with similar source

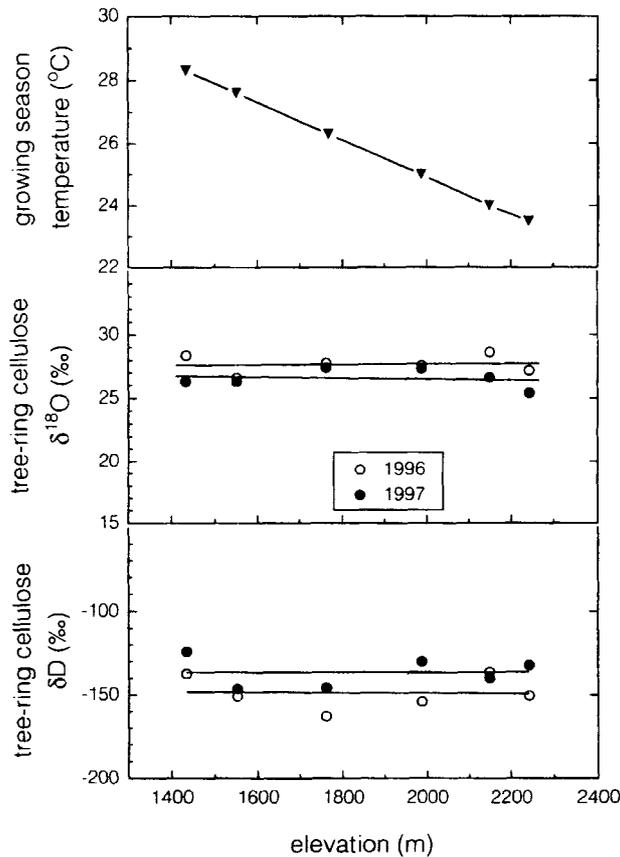


FIGURE 5 The variation in the oxygen and hydrogen isotopic composition in tree-ring cellulose as a function of elevation for two growing seasons along with the mean daytime temperature as calculated from the adiabatic lapse rate ($0.006^{\circ}\text{C m}^{-1}$). Cellulose values are means ($n = 2$).

and leaf water δD and $\delta^{18}O$ values. Although the variation in the leaf water values associated with seasonal environmental effects were substantial (see also Fig. 3), all sites experienced similar environmental variation and thus any site differences in the δD and $\delta^{18}O$ of tree-ring cellulose cannot be attributed to differences in leaf water evaporative enrichment.

Figure 5 shows the mean growing season temperature for each site as calculated using the adiabatic lapse rate and the mean temperature measured at 1987 m. The 810 m elevational transect produced a $5^{\circ}C$ range in growing season temperature based on an adiabatic lapse rate of $6^{\circ}C km^{-1}$. This lapse rate may actually be rather conservative since data from weather stations located at two elevations in this same canyon indicate that the growing season lapse rate might be closer to $9^{\circ}C km^{-1}$ which would produce a greater than $7^{\circ}C$ range in temperature over the transect. Regardless of which lapse rate was utilized, the temperature range across the elevational transect did not produce a significant variation in the δD and $\delta^{18}O$ values in tree ring cellulose (Fig. 5). The slopes of the regressions were not significantly different from zero ($p > 0.5$) and the highest r^2 was 0.036, implying that elevation (or temperature) can explain, at best, less than 4% of the variation in the isotopic composition of tree-ring cellulose. The slight differences in offset between years may be associated with environmental differences, however, the differences were not statistically significant ($p > 0.5$) for either δD or $\delta^{18}O$.

DISCUSSION

Using riparian trees along an elevational transect provided an appropriate test of possible temperature-dependent biological fractionation during cellulose synthesis. These small cottonwood trees utilized the river as a water source as seen by the similarity in isotope ratio values of stem and stream waters (Figs. 1–3). The utilization of stream water reduces the seasonal and elevational variability in source water δD and $\delta^{18}O$ that is often observed in meteoric and soil waters [10, 35, 36]. For example, Burk and Stuiver [10] observed $\delta^{18}O$ values of cellulose along an elevational transect on Mount Rainer in Washington State to infer temperature dependence in tree-ring cellulose of $0.41\text{‰ }^{\circ}C^{-1}$. However,

they were unable to reach any conclusions regarding temperature effects on biochemical fractionation since the changes in elevation/temperature were correlated with changes in the $\delta^{18}\text{O}$ of precipitation and thus source water. In this study, the relatively constant water source and environmental conditions (*e.g.*, vapor pressure) over the short transect distance (approx 13 km) created similar leaf water δD and $\delta^{18}\text{O}$ values across sites (Figs. 1, 2 and 4). The biochemical steps from leaf water to tree-ring cellulose involve both autotrophic and heterotrophic fractionation factors [18–20] as well as isotopic exchange with medium water [12, 13, 20]. Thus, if leaf and stem waters are constant over the elevation/temperature transect, then any differences in cellulose δD and $\delta^{18}\text{O}$ values across the transect should be a result of temperature effects on biochemical fractionation factors or the proportion of isotopic exchange with xylem water during cellulose synthesis or both.

There were no distinguishable trends associated with elevation and the δD or $\delta^{18}\text{O}$ in tree-ring cellulose indicating that neither biochemical fractionation factors nor the proportion of isotopic exchange are strongly temperature dependent. In models that describe leaf water evaporative enrichment [33, 34], the equilibrium fractionation factor is temperature dependent [37] and may effect cellulose δD and $\delta^{18}\text{O}$ values even when trees are utilizing an isotopically constant water source. However, in this study, leaf water δD and $\delta^{18}\text{O}$ values were monitored throughout the growing season and did not show significant elevational trends that might mask the temperature dependence of biological fractionation during cellulose synthesis.

Previous studies have estimated a potential temperature-dependent fractionation factor based mostly on residual analyses. White [38] estimated a temperature effect of $+1.6\text{‰}\text{°C}^{-1}$ for pine by modeling leaf water δD and measured tree-ring cellulose. Yet these results were not constrained by complete measurements of the environmental parameters which influence leaf water enrichment. Later White *et al.* [4] estimated the temperature effects on biological fractionation in woody plants by subdividing the annual growth ring of white pine and determining the time of year that each intra-ring section was produced. The seasonal temperature variation was then correlated with each intra-ring section. Using a model to calculate leaf water δD values and measured source water δD values they calculated a net biological

fractionation. The variability in the values of net biological fractionation calculated was explained by differences in ambient temperature and the effect was estimated to be $+1.3\text{‰}\text{°C}^{-1}$. White *et al.* [4] found that if they used temperature estimates from early morning and evening rather than at midday, the temperature effect of biological fractionation became nonsignificant. Buhay *et al.* [39] showed that the temperature dependence in the data of White *et al.* [4] could be reconciled by invoking a temperature dependent depletion of leaf water that acts in opposition to evaporative enrichment. However, a mechanism for such a temperature dependent depletion of leaf water δD is not established as yet [39]. Still other studies have used aquatic plants [2, 23] which are not directly applicable to terrestrial plants because of differences in leaf water enrichment.

In this study, not only did we maintain a constant water source across the elevation/temperature transect, but our analysis did not depend on modelling leaf water δD and $\delta^{18}\text{O}$ values, since these parameters were measured through the growing season. In addition, temperature differences are not dependent on when the cellulose was laid down since the trees at different elevations will always be experiencing different temperatures due to lapse rate effects. From our data, the temperature dependence of biochemical fractionation was not statistically significant, contrasting with results from other studies [2, 4, 23]. Our results indicate that temperature may be at best a minor factor influencing biochemical fractionation in cellulose synthesis. It is certainly much less important than direct effects of temperature on the δD and $\delta^{18}\text{O}$ of meteoric waters or leaf water evaporative enrichment. If the temperature effects on biochemical fractionation are minimal as we conclude, then the interpretation of the hydrogen and oxygen isotope ratios in tree-ring cellulose become simplified.

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