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Geochimica et Cosmochimica Acta 71 (2007) 2463–2473
Geochimica et Cosmochimica Acta
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# Oxygen isotope ratios of cellulose-derived phenylglucosazone: An improved paleoclimate indicator of environmental water and relative humidity

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Received 24 October 2006; accepted in revised form 13 March 2007; available online 19 March 2007

**Abstract**

Oxygen atoms within fossil wood provide high-resolution records of climate change, particularly for the Quaternary. However, current analysis methods of fossil cellulose do not differentiate between different positions of the oxygen atoms. Here, we propose a refinement to tree-cellulose paleoclimatology modeling, using the cellulose-derived compound phenylglucosazone as the isotopic substrate. Stem samples from trees were collected at northern latitudes as low as 24°37′N and as high as 69°00′N. We extracted stem water and cellulose from each stem sample and analyzed them for their $^{18}O$ content. In addition, we derived the cellulose to phenylglucosazone, a compound which lacks the oxygen attached to the second carbon of the cellulose–glucose moieties. Oxygen isotope analysis of phenylglucosazone allowed us to calculate the $^{18}O$ content of the oxygen attached to the second carbon of the cellulose–glucose moieties. By way of these analyses, we tested two hypotheses: first, that the $^{18}O$ content of the oxygen attached to second carbon will more closely reflect the $^{18}O$ content of the stem water, and will not resemble the $^{18}O$ content of either cellulose or its derivative phenylglucosazone. Second, tree-ring models that incorporate the variable oxygen isotope fractionation shown here and elsewhere are more accurate than those that do not. Our first hypothesis was rejected on the basis that the oxygen isotope ratios of the oxygen attached to the second carbon of the glucose moieties had a noisy isotopic signal with a large standard deviation and gave the poorest correlation with the oxygen isotope ratios of stem water. Related to this isotopic noise, we observed that the correlation between oxygen isotope ratios of phenylglucosazone with both stem water and relative humidity were higher than those observed for cellulose. Our hypothesis about tree-ring models which account for changes in the oxygen isotopic fractionation during cellulose synthesis was consistent only for the $^{18}O$ content of phenylglucosazone. We showed that the tree-ring model based on the $^{18}O$ content of phenylglucosazone was an improvement over existing models that are based on whole cellulose. Additionally, this approach may be used in other cellulose based archives such as peat deposits and lacustrine sediments.


## 1. INTRODUCTION

Cellulose is chronologically layered in tree rings and can be highly preserved; therefore, it has successfully been used as an isotopic recorder of paleoclimate and paleohydrology. Our knowledge of meteorological and hydrological processes that alter the isotopic composition of plant available water and its modification within the plant thereof is extensive (Craig and Gordon, 1965; Dongmann et al., 1974; Farris and Strain, 1978; Flanagan et al., 1991; Luo et al., 1992; Wang and Yakir, 1995; Fricke and O'Neil,

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doi:10.1016/j.gca.2007.03.004

1999; Barbour and Farquhar, 2003 and several others). The next step towards using this climate/hydrology isotopic proxy is the understanding of how the oxygen and hydrogen isotope ratios of water in the different plant compartments leaf and stem contribute to the actual oxygen and hydrogen isotope ratios of cellulose as it is layered in the tree-ring structure. One recent relevant finding is that there are post-photosynthetic reactions in which ~40% of the oxygen of the cellulose substrate, as it is translocated to the tree trunk, is re-imprinted with the isotopic signature of tree trunk water. This finding has been confirmed in model biological systems, in actual hydroponic tree growth experiments (Sternberg et al., 1986; Yakir and DeNiro, 1990; Luo et al., 1992; Roden and Ehleringer, 1999) and has been elegantly incorporated in a mechanistic tree-ring isotope model (Roden et al., 2000). As yet there is no way to disentangle the isotopic signature of cellulose derived from leaf-water (known as the autotrophic signal, which is sensitive to relative humidity) and the isotopic signature which is derived from the tree trunk or stem water (known as the heterotrophic signal, which is sensitive to temperature or hydrological processes). Our knowledge of biochemical processes during cellulose synthesis is still at the "black-box" stage, where it is assumed that oxygen isotope fractionation during cellulose synthesis is 27‰ relative to the source water. However, this is an average and actual values can vary by as much as 15% (DeNiro and Epstein, 1981; Sternberg, 1989b). Further, it has been assumed that this fractionation is invariable with respect to the metabolic pathway leading to cellulose synthesis and the oxygen location in the glucose moieties of cellulose.

Recently, we developed a technique which allows us to measure the $\delta^{18}O$ value of the oxygen attached to the second carbon of the glucose moieties separate from the oxygen attached to carbon 3–6 of the same moieties (Sternberg et al., 2003, 2006). Our most recent findings show that: (1) 70% of the oxygen's attached to the second carbon of the glucose moieties can exchange with trunk/stem water before it is incorporated into cellulose, (2) the metabolic pathway leading to sucrose and on to cellulose synthesis can influence the final $\delta^{18}O$ value of the cellulose molecule and (3) there are different isotopic fractionations associated with oxygen attached to different positions in the glucose moieties (Sternberg et al., 2006). The two latter observations indicate that there might be differences in the fractionation during autotrophic and heterotrophic cellulose synthesis.

The above findings lead us to two hypotheses which we test here. First, since the oxygen attached to the second carbon of the cellulose–glucose moieties may gain up to 70% of its oxygen isotope label from trunk water, we hypothesize that the $\delta^{18}O$ value of the oxygen attached to the second carbon of the cellulose–glucose moieties should be more closely correlated with stem water compared with the $\delta^{18}O$ value of the whole cellulose molecule. Second, and related to the second and third findings above, we hypothesize that models predicting the isotopic composition of tree-ring cellulose or its derivative thereof might be improved if we allow for variations in the isotopic fractionations during autotrophic and heterotrophic cellulose synthesis. To test the first hypothesis, we gathered stem samples from several latitudes in the northern hemisphere and compared the $\delta^{18}O$ values of cellulose and derivatives thereof with that of stem water. To test the second hypothesis, we compared a statistically best-fit equation between $\delta^{18}O$ value of cellulose or its derivative and $\delta^{18}O$ values of stem water and RH with equations generated by two mechanistic models. The first model assumes an invariable oxygen isotopic fractionation during cellulose synthesis, whereas the second assumes variable oxygen isotopic fractionations during cellulose synthesis.

## 2. MATERIALS AND METHODS

### 2.1. Stem water and cellulose extraction

Well suberized stem samples, approximately 0.75 cm in diameter and 4–5 cm in length, were collected at various locations during the growing season as well as donated by co-investigators and colleagues (Table 1). These samples covered a range of latitudes from 24°37′N (Florida, USA) to 69°00′N (Siberia, RUS). Stem samples were debarked and placed in a sealed tube and taken to the laboratory for water distillation according to the method of Moreira et al. (1997). After distillation stem samples were ground in a Wiley mill to pass through a 25 mesh and cellulose extracted according to the method of Sternberg (1989a). A small aliquot of the cellulose was saved for oxygen isotope analyses and 0.3 g of cellulose was used for hydrolysis and phenylglucosazone synthesis.

### 2.2. Cellulose hydrolysis and derivation

Cellulose was hydrolyzed with 99% Trifluoroacetic acid (TFA) according the method of Fengel and Wegener (1979). Cellulose (0.3 g) was soaked in 48 ml of TFA for a period of 2 h, after which it was boiled in a refluxer for a period of 15 min. De-ionized Water (18 ml) was added to the boiling solution and refluxing continued for a period of 15 min. A further aliquot of water (150 ml) was added and the solution refluxed for another 30 min. The solution was filtered through a glass-fiber filter after the final refluxing and the filtrate roto-evaporated to a thick syrupy consistency. About 10 ml of water was added to this thick syrupy residue and the solution was roto-evaporated again, after which it was placed in a freeze dryer before derivation.

The cellulose hydrolysate was derived to phenylglucosazone according to the method of Oikawa et al. (1998). Four point two ml of a 12% acetic acid solution was added to the above hydrolysate with 1 ml of phenylhydrazine. This mixture was mixed with a stir bar covered and placed in a boiling water bath for a period of 2 h, after which it was placed in a refrigerator until cool and filtered through a fritted glass filter (Kimax, 15 ml, 20 C). The filtrand was soaked in hexane for a period of 15 min and rinsed with distilled water. The phenylglucosazone was scraped from the fritted glass filter, freeze dried, washed with 95% methanol and freeze dried again.

Table 1
Species, location, average growing season relative humidity, latitude and $\delta^{18}O$ values of stem water, stem cellulose, its derivative phenylglucosazone and oxygen in the second carbon of the cellulose–glucose moieties for several stems collected at various locations during the growing season

| Species | Location | RH | Latitude | $\delta^{18}O$ values | | | |
|---|---|---|---|---|---|---|---|
| | | | | Stem Water | Cellulose | P–G | C-2 Oxy. |
| *Manilkara bahamensis* | Sugarloaf Key FL/USA | 0.68 | 24°37′N | −0.9 | 28.9 | 29.9 | 24.6 |
| *Rhizophora mangle* | Sugarloaf Key FL/USA | 0.68 | 24°37′N | 0.8 | 25.9 | 31.7 | 2.6 |
| *Conocarpus erectus* | Sugarloaf Key FL/USA | 0.68 | 24°37′N | −0.4 | 26.7 | 30.4 | 12.0 |
| *Pinus elliottii* | Sugarloaf Key FL/USA | 0.68 | 24°37′N | −1.0 | 29.0 | 27.4 | 35.7 |
| *Picea schrerkiana* | Wageningen NL | 0.75 | 51°58′N | −8.2 | 29.7 | 24.4 | 50.9 |
| *Viburnum sieboldi* | Wageningen NL | 0.75 | 51°58′N | −7.0 | 29.3 | 25.2 | 45.9 |
| *Prunus serrulaea* | Wageningen NL | 0.75 | 51°58′N | −7.4 | 29.4 | 26.5 | 41.1 |
| *Populus* sp. | Vienna AUT | 0.59 | 48°14′N | −11.1 | 25.4 | 17.1 | 58.5 |
| *Betula* sp. | Vienna AUT | 0.59 | 48°14′N | −11.2 | 26.3 | 20.4 | 49.7 |
| *Sambucus nigra* | Vienna AUT | 0.59 | 48°14′N | −12.4 | 29.0 | 24.8 | 45.9 |
| *Cytisus scoparius* | Berkeley CA/USA | 0.44 | 37°52N | −6.2 | 31.0 | 25.8 | 51.8 |
| *Quercus* sp. | Berkeley CA/USA | 0.44 | 37°52N | −6.9 | 29.3 | 28.0 | 34.6 |
| *Aesculus californica* | Berkeley CA/USA | 0.44 | 37°52N | −6.1 | 28.9 | 27.9 | 32.7 |
| *Pinus flexilis* | Laramie WY/USA | 0.43 | 41°19′N | −13.1 | 24.1 | 21.3 | 35.0 |
| *Cercocarpus montanus* | Laramie WY/USA | 0.43 | 41°19′N | −13.2 | 25.1 | 20.3 | 44.5 |
| *Cercocarpus montanus* | Laramie WY/USA | 0.43 | 41°19′N | −12.5 | 27.6 | 21.6 | 51.8 |
| *Eucalyptus* sp. | Santa Barbara/CA/USA | 0.68 | 34°25′N | −4.8 | 30.0 | 24.9 | 50.5 |
| *Quercus chrysolepsis* | BueltonCA/USA | 0.66 | 34°37′N | −5.1 | 32.7 | 28.1 | 51.2 |
| *Quercus chrysolepsis* | Salinas CA/USA | 0.71 | 36°40′N | −5.2 | 30.3 | 24.6 | 53.1 |
| *Pinus radiata* | Santa Cruz CA/USA | 0.71 | 36°58′N | −5.2 | 29.4 | 25.9 | 43.3 |
| *Populus fremontii* | Sacramento CA/USA | 0.48 | 38°34′N | −10.0 | 28.6 | 24.2 | 46.1 |
| *Pseudotsuga menziesii* | Hw 50 Tahoe CA/USA | 0.49 | 38°49′N | −10.1 | 27.5 | 21.1 | 53.3 |
| *Pinus ponderosa* | Hw 50 Tahoe CA/USA | 0.49 | 38°49′N | −12.2 | 25.9 | 22.7 | 38.6 |
| *Pinus jeffreyi* | East Sierra CA/USA | 0.33 | 39°06′N | −12.3 | 27.7 | 22.0 | 50.6 |
| *Pinus radiata* | San Francisco CA/USA | 0.67 | 37°47′N | −13.6 | 26.1 | 23.4 | 36.8 |
| *Quercus imolina* | Archibold FL/USA | 0.75 | 27°06′N | −5.3 | 28.9 | 23.6 | 50.4 |
| *Quercus germinata* | Archibold FL/USA | 0.75 | 27°06′N | −4.4 | 30.0 | 25.5 | 47.9 |
| *Rhizophora mangle* | Everglades FL/USA | 0.68 | 25°19′N | −1.5 | 31.0 | 28.4 | 41.0 |
| *Larix sibirica* | Cherskii, Siberia, RUS | 0.71 | 69°00′N | −19.1 | 19.1 | 15.6 | 33.0 |
| *Larix sibirica* | Cherskii, Siberia, RUS | 0.71 | 69°00′N | −19.8 | 18.2 | 13.6 | 36.7 |
| *Picea mariana* | Delta Junction AK/USA | 0.51 | 63° 53′N | −18.1 | 21.7 | 16.5 | 42.4 |
| *Picea mariana* | Delta Junction AK/USA | 0.51 | 63° 53′N | −17.0 | 21.7 | 19.6 | 29.9 |
| *Populus tremuloides* | Delta Junction AK/USA | 0.51 | 63°55′N | −15.0 | 18.8 | 17.4 | 24.1 |
| *Populus tremuloides* | Delta Junction AK/USA | 0.51 | 63°55′N | −15.8 | 19.0 | 17.2 | 26.3 |
| *Osmanthus* sp. | Tucson AZ/ USA | 0.26 | 32°07′N | −8.7 | 31.7 | 31.8 | 31.1 |
| *Pinus* sp. | Tucson AZ/ USA | 0.26 | 32°07′N | −9.3 | 30.4 | 29.0 | 36.1 |
| *Pinus pinea* | Tucson AZ/ USA | 0.26 | 32°07′N | −9.1 | 30.2 | 29.2 | 34.2 |
| *Quercus Virginiana* | Tucson AZ/ USA | 0.26 | 32°07′N | −7.1 | 30.2 | 33.6 | 16.4 |
| *Ceratonia Siliqua* | Tucson AZ/ USA | 0.26 | 32°07′N | −8.3 | 28.8 | 33.9 | 8.1 |

### 2.3. Isotopic analysis

Oxygen isotope ratios of stem water was done by equilibrating $CO_2$ with water for a period of 48 h at 25 °C and then extracting the $CO_2$ for oxygen isotope determination. This process was done by using a multiflow system connected to an Isoprime isotope ratio mass spectrometer (GV, Manchester, UK). Oxygen isotope ratios of cellulose and phenylglucosazone were determined as previously (Sternberg et al., 2003). All oxygen isotopic abundances are reported here in δ(‰) units, relative to Vienna Standard Mean Ocean Water (VSMOW) and are given by

$$\delta^{18}O = \left[\frac{R_{sample}}{R_{vsmow}} - 1\right] \cdot 1000. \quad (1)$$

$R_{sample}$ and $R_{vsmow}$ represent the molar ratio of $^{18}O/^{16}O$ of the sample and Vienna-based standard mean ocean water. The precision of analysis was ±0.36‰ for the organic oxygen and ±0.1‰ for the water samples. The $\delta^{18}O$ value of the oxygen attached to the second carbon was calculated as previously (Sternberg et al., 2003):

$$\delta^{18}O_{C\text{-}2} = (5 \cdot \delta^{18}O_{cell}) - (4 \cdot \delta^{18}O_{P\text{–}G}), \quad (2)$$

having $\delta^{18}O_{C\text{-}2}$, $\delta^{18}O_{cell}$ and $\delta^{18}O_{P\text{–}G}$ as the $\delta^{18}O$ values of the oxygen attached to the second carbon of the glucose moieties, of cellulose and of the phenylglucosazone derivative, respectively.

### 2.4. Data analysis

We used latitude (LAT) and altitude (ALT) of the location of each sample to calculate the expected isotopic composition of the mean annual amount-weighted precipitation

($\delta^{18}O_{ppt}$) using the Bowen–Wilkinson equation (Bowen and Wilkinson, 2002):

$$\delta^{18}O_{ppt} = -0.0051 \cdot (|LAT|)^2 + 0.1805 \cdot (|LAT|) - 0.002 \cdot (ALT) - 5.247. \quad (3)$$

We compared the expected $\delta^{18}O$ value of precipitation at each location with that observed for stem water ($\delta^{18}O_{sw}$). We used the latitude and longitude of each sample to determine the growing season (April–September) average daytime relative humidity of the nearest station from the International Station Meteorological Climate Summary, version 4.0, September 1996 (Federal Climate Complex, Asheville, North Carolina). In some cases a station did not have hourly relative humidity, in which case we used the average for the above months.

Model II regression analysis was performed for the relationship between $\delta^{18}O_{cell}$ or $\delta^{18}O_{P-G}$ versus $\delta^{18}O_{sw}$ as the only independent variable with the exclusion of samples from Arizona which had cellulose synthesized under very low RH (~26%). We excluded the Arizona samples since its low relative humidity can cause extremely high leaf water $\delta^{18}O$ values which subsequently affects $\delta^{18}O_{cell}$. A model II regression was used because there are measuring errors associated with both the dependent and independent variables (Sokal and Rohlf, 1995).

When we considered both $\delta^{18}O_{sw}$ and RH as independent variables determining either $\delta^{18}O_{cell}$ or $\delta^{18}O_{P-G}$, we used a standard multiple linear regression analysis to derive the relationship between $\delta^{18}O_{cell}$ or $\delta^{18}O_{P-G}$ values as a function of $\delta^{18}O_{sw}$ and relative humidity (RH). We adjusted the coefficients of the multiple linear regressions so that the relationship between the observed $\delta^{18}O_{cell}$ or $\delta^{18}O_{P-G}$ and predicted ($\delta^{18}O^{p}_{cell}$ or $\delta^{18}O^{p}_{P-G}$, respectively) values had a slope of 1 and an intercept at 0. We first derived the multiple linear regression equation with the respective coefficients

$$\delta^{18}O^{p}_{cell}(\text{or } \delta^{18}O^{p}_{P-G}) = a_0 + a_1 \cdot RH + a_2 \cdot \delta^{18}O_{sw}. \quad (4)$$

We regressed the $\delta^{18}O_{cell}{}^{p}$ or $\delta^{18}O_{P-G}{}^{p}$ values with the respective values of the observed components by a simple linear regression

$$\delta^{18}O^{p}_{cell}(\text{or } \delta^{18}O^{p}_{P-G}) = m \cdot \delta^{18}O_{cell}(\text{or } \delta^{18}O_{P-G}) + b, \quad (5)$$

in which $m$ and $b$ are the respective slope and intercept of the linear regression. We then calculated the adjusted coefficients to

$$A_0 = \frac{a_0 - b}{m}, \quad A_1 = \frac{a_1}{m} \quad \text{and } A_2 = \frac{a_2}{m}. \quad (6)$$

We compared the results of the modeled $\delta^{18}O_{cell}$ and $\delta^{18}O_{P-G}$ value with that predicted by the above best-fit equation with a paired $T$ test between residuals generated by the model with those generated by the best-fit equation.

## 3. RESULTS

The observed $\delta^{18}O_{sw}$ values were highly correlated ($r = 0.89$, $P < 0.01$) with those of precipitation predicted by the Bowen–Wilkinson equation (Bowen and Wilkinson, 2002) having a regression slope of 1.15 (Fig. 1). Both cellulose and phenylglucosazone $\delta^{18}O$ values were highly correlated with the $\delta^{18}O$ values of stem water ($r = 0.77$, $P < 0.05$ and $r = 0.91$, $P < 0.05$, respectively) when the Arizona samples were not included in the analysis and described by the following linear equations:

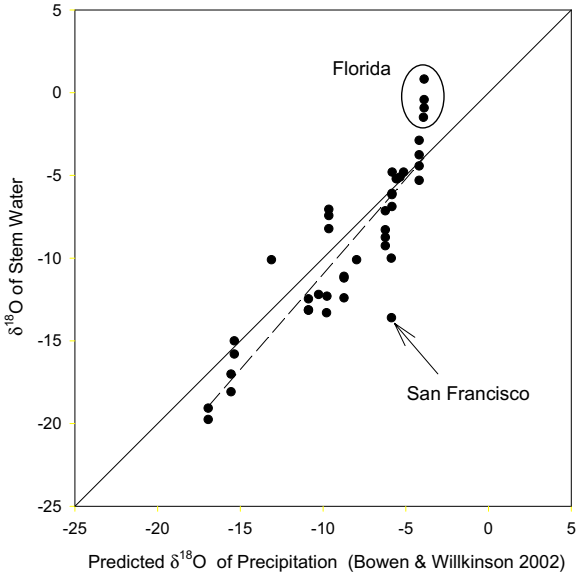


Fig. 1. Observed $\delta^{18}O$ values of stem water versus those of precipitation predicted by the Bowen and Wilkinson equation (Bowen and Wilkinson, 2002); stippled line is for observed $\delta^{18}O_{sw} = (1.15^{*}\ \delta^{18}O_{ppt}) + 0.55$, $r^2 = 0.79$, while solid line represents a one to one relationship.

$$\delta^{18}O_{cell} = (0.52 \cdot \delta^{18}O_{sw}) + 31.6. \quad (7)$$

$$\delta^{18}O_{P-G} = (0.73 \cdot \delta^{18}O_{sw}) + 30.1. \quad (8)$$

No significant correlation between the $\delta^{18}O$ value of oxygen attached to the second carbon of the glucose moieties and stem water was observed ($r = 0.14$, $P > 0.05$).

The relationship between $\delta^{18}O_{cell}$ values and those of stem water was better fit by a curvilinear equation ($r = 0.89$, $P < 0.01$) which approaches the asymptotic value of 30‰ beginning at the $\delta^{18}O_{sw}$ value of −10‰ (Figs. 2 and 3). Although the cellulose in the Arizona samples was produced under very low relative humidity, their $\delta^{18}O_{cell}$ values were only slightly higher than those of cellulose generated in stems from the Netherlands, having stem water with the similar oxygen isotope ratios and higher relative humidity (Table 1). Cellulose samples which had low $\delta^{18}O$ values relative to those predicted by $\delta^{18}O_{sw}$ with Eq. (7), or those expected based on the RH had relatively low $\delta^{18}O_{C\text{-}2}$ values (Fig. 2a and c). For example, when the oxygen attached to the second carbon of the cellulose–glucose moieties from the Arizona samples were removed, the $\delta^{18}O$ values of the remaining oxygen ($\delta^{18}O_{P-G}$) were substantially greater than that predicted by the regression line derived by only using samples from higher humidity sites (Eq. (8), Fig. 2b). The correlation of $\delta^{18}O_{cell}$ and $\delta\ ^{18}O_{P-G}$ on $\delta^{18}O_{sw}$ and RH values were both significant ($r = 0.781$, $P < 0.01$ and $r = 0.895$, $P < 0.01$, respectively) and gave the following equations after adjusting the coefficients:

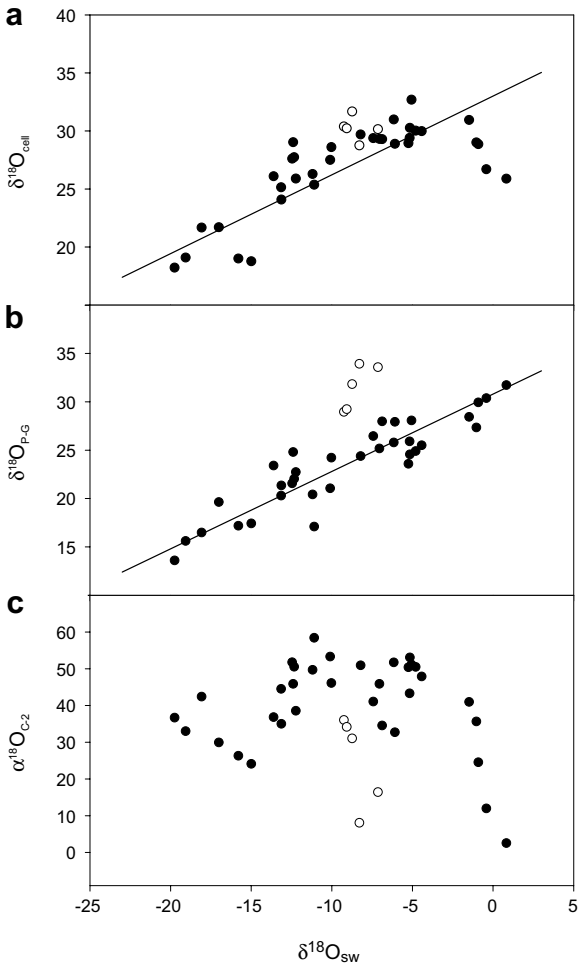


Fig. 2. Oxygen isotope ratios of stem cellulose (a), phenylglucosazone (b), and oxygen attached to the second carbon of the cellulose–glucose moieties (c) versus the $\delta^{18}O$ values of stem water. Empty circles represent values of cellulose for stems collected in Tucson, Arizona where the relative humidity is exceptionally low. Regression lines are for all samples except for the Tucson, Arizona samples. Regression lines are: $\delta^{18}O_{cell} = (0.52*\delta\ ^{18}O_{sw}) + 31.6$ with $r^2 = 0.58$ and $P < 0.05$, $\delta\ ^{18}O_{P-G} = (0.73*\delta^{18}O_{sw}) + 30.1$ with $r^2 = 0.83$ and $P < 0.05$, and not shown $\delta\ ^{18}O_{C-2} = (-0.30*\delta^{18}O_{sw}) + 37.6$ with $r^2 = 0.02$ and $P > 0.05$.

$$\delta^{18}O^{P}_{cell} = 41.73 - (10.63 \cdot RH) + (0.948 \cdot \delta^{18}O_{sw}). \quad (9)$$

$$\delta^{18}O^{P}_{P-G} = 43.87 - (17.47 \cdot RH) + (1.076 \cdot \delta^{18}O_{sw}). \quad (10)$$

## 4. DISCUSSION

### 4.1. Isotopic composition of stem water relative to that predicted for precipitation water

The $\delta^{18}O$ values of stem water were significantly related to those of the amount-weighted mean annual precipitation predicted by the Bowen and Wilkinson (2002) equation (Fig. 1). The observed deviations are expected since Eq. (3) predicts the oxygen isotope ratios of precipitation whereas the stem water is determined by the oxygen isotope ratios of soil water, which can be modified relative to that of precipitation by evaporation and the soil recharge patterns. For example, the stem water samples from Florida (Fig. 1) probably represent water uptake from surface water which has undergone isotopic enrichment during evaporation. The $\delta^{18}O_{sw}$ value of the San Francisco sample (Fig. 1) is much lower relative to that predicted by Eq. (3) and it is possibly caused by the fact that much of the water in urban areas of California is derived from high altitude sources in the Sierra Nevada. Many of the $\delta^{18}O_{sw}$ values were lower than that predicted by the Eq. (3) (Fig. 1). This could be caused by the available soil water not completely integrating the isotopic ratios of precipitation by a simple weighed average, which was used to derive Eq. (3) (Bowen and Wilkinson, 2002).

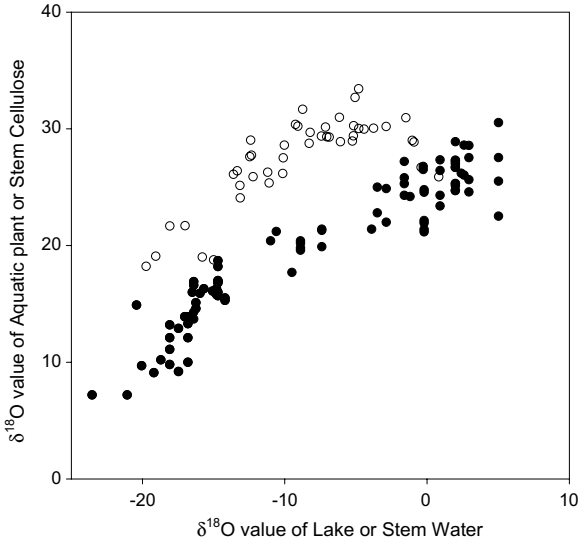


Fig. 3. Oxygen isotope ratio of stem cellulose versus that of stem water (empty circles) and $\delta^{18}O$ value of aquatic plant cellulose versus that of their respective lake water (full circles) from different studies (Sternberg, 1988; Anderson, 2000; Sauer et al., 2001).

### 4.2. Correlation between $\delta^{18}O$ values of the organic fractions and stem water

Our expectation of a high correlation between $\delta^{18}O_{C-2}$ and $\delta^{18}O_{sw}$ values was not observed. To the contrary, the correlation was the poorest of all the organic components measured here and not significant (Fig. 2c). This poor correlation is in contrast to two of our previous studies with laboratory seed germination experiments showing a highly significant correlation between $\delta^{18}O_{C-2}$ and $\delta^{18}O$ values of the available water (Sternberg et al., 2003, 2006). We hypothesize that the high species and environmental variability in the field samples shown here may be adding the isotopic noise to the $\delta^{18}O_{C-2}$ value. The average $\delta^{18}O_{C-2}$ enrichment relative to that of the respective stem water was $47.5 \pm 14.9‰$ and similar to that previously observed: $48.8 \pm 20.0‰$ for cellulose synthesized from starch (Sternberg et al., 2003) and $51.0 \pm 5.0‰$ for cellulose synthesized from lipid reserves (Sternberg et al., 2006). However, this

isotopic signal has a high standard deviation and is probably responsible for lowering the correlation between $\delta^{18}O_{cell}$ versus $\delta^{18}O_{sw}$ values. When this signal is removed, as cellulose is derived to phenylglucosazone, and when samples from a low RH site are excluded from the regression analysis, the correlation between the $\delta^{18}O_{P-G}$ and $\delta^{18}O_{sw}$ increases considerably (Fig. 2b).

The lower correlation between stem cellulose and water seems to be caused by two mechanisms: one that drastically lowers the oxygen isotope ratios of the oxygen attached to the second carbon and one causing a gradual decrease in the oxygen isotopic fractionation as the $\delta^{18}O_{sw}$ increases (Fig. 2a). Recently, we have shown that the isotopic history of the metabolic pathway leading to sucrose and on to cellulose is robust enough to show a difference in the final cellulose oxygen isotope ratio (Sternberg et al., 2006). We note that samples which showed lower $\delta^{18}O_{C-2}$ values (<35‰) were either exposed to higher salinities (Florida samples), low RH (Arizona samples) or low temperatures (Arctic samples), which are all abiotic factors that can cause a plant to generate simple carbohydrates, alcohols and amino acid to adjust osmotically or provide membrane protection (Bohnert et al., 1995). We hypothesize that these plants probably undergo shifts in carbohydrate metabolism which are recorded in the oxygen isotope ratios of cellulose. We are not aware of any mechanism which might cause the gradual decrease in the fractionation with the increase in the $\delta^{18}O$ value of the source water. We are certain, however, that this effect is not caused by a leaf water isotopic enrichment phenomenon since it parallels that which is observed in cellulose of submerged aquatic plants relative to the isotopic composition of lake water (Fig. 3). Recent studies indicate a persistent metabolic water pool in bacterial cultures, which presumably would have a constant $\delta^{18}O$ value indicative of atmospheric oxygen and any fractionation associated with its reduction during respiration (Kreuzer-Martin et al., 2005). This isotopically constant pool of water, if it also exists in plant cells, could buffer increases in the isotopic ratios of intracellular water from increases in the isotopic ratios of environmental water and therefore cause a decoupling between the $\delta^{18}O_{cell}$ value and those of environmental water.

The relatively high correlation between $\delta^{18}O_{P-G}$ and $\delta^{18}O_{sw}$ values indicates that this cellulose derivative is a potential proxy to determine the isotopic composition of water available to plants when very low RH samples are not included. This and a previous attempt (Epstein et al., 1977) to correlate $\delta^{18}O$ values of stem cellulose to those of stem or ambient water gave a lower correlation ($r = 0.77$ and $r = 0.81$, respectively) compared to that based on $\delta^{18}O_{P-G}$ versus $\delta^{18}O_{sw}$ ($r = 0.91$). We note that the relationship between $\delta^{18}O_{cell}$ values and those of ambient water reported by Epstein et al. (1977) showed the same asymptotic pattern observed here beginning at ambient water $\delta^{18}O$ values of −10‰. Approximately, 83% of the variance in the oxygen isotopic composition of the stem water is recorded in the respective variance of phenylglucosazone, providing that the RH was not extremely low during cellulose synthesis. For example, Eocene Arctic cellulose samples having $\delta^{18}O_{P-G}$ values ranging from 16.7‰ to 18.8‰, when applied to the back calculation of the above regression equation, predicts Eocene source water $\delta^{18}O$ values in the range of −18.4‰ to −15.5‰ (Table 2), similar to the range of −17.9‰ to −13.6‰ extrapolated by other methods (Jahren et al., 2002, 2003).

### 4.3. The best-fit multiple linear regression between $\delta^{18}O$ values of organic components and that of stem water and RH

A multiple linear regression analyses of the $\delta^{18}O$ values of cellulose or phenylglucosazone as a function of stem water and relative humidity both gave significant results. The phenylglucosazone gave a higher correlation coefficient with the ability to explain 80% of the variance in $\delta^{18}O_{P-G}$ from the isotopic values of source water and relative humidity as opposed to 61% for cellulose $\delta^{18}O$ values (Fig. 4). The sample set presented here is composed of a broad taxonomic and functional spectrum of trees ranging from mangroves to pine trees. We hypothesize that a more narrowly defined taxonomic group would give an even higher correlation coefficient. For example, $r^2$ increases to 0.85 if we only include gymnosperms from our sample set. Although the ability to predict the $\delta^{18}O_{P-G}$ value based on $\delta^{18}O_{sw}$ and RH values has improved over that of cellulose, it still leaves us with the uncertainty of whether the derivative $^{18}O$ enrichment is caused either by high temperatures or hydrological factors causing the source water to be isotopically enriched (Fricke and O'Neil, 1999) and/or low relative humidity causing the leaf water to be isotopically enriched and transferring this signal via the translocation of isotopically enriched carbohydrates to stem cellulose (Roden et al., 2000).

### 4.4. Mechanistic models that replicate the best-fit multiple linear regression equation

#### *4.4.1. The constant fractionation model*

Can we replicate the best-fit linear Eqs. (9) and (10) having three parameters: $A_0$ which is a constant, $A_1$ which is multiplied by the relative humidity and $A_2$ which is multi-

Table 2
Predicted $\delta^{18}O$ values of stem water based on $\delta^{18}O_{P-G}$ values and Eq. (8) for Eocene Metasequoia fossil cellulose samples from the Axel Heiberg Island

| Sample ID | $\delta^{18}O$ value of cellulose | $\delta^{18}O$ value of phenylglucosazone | Predicted $\delta^{18}O$ value of stem water |
|---|---|---|---|
| 102 | 19.7 | 17.6 | −17.1 |
| 030A | 19.4 | 18.8 | −15.5 |
| 030B | 20.5 | 18.6 | −15.7 |
| 034A | 18.2 | 16.7 | −18.4 |

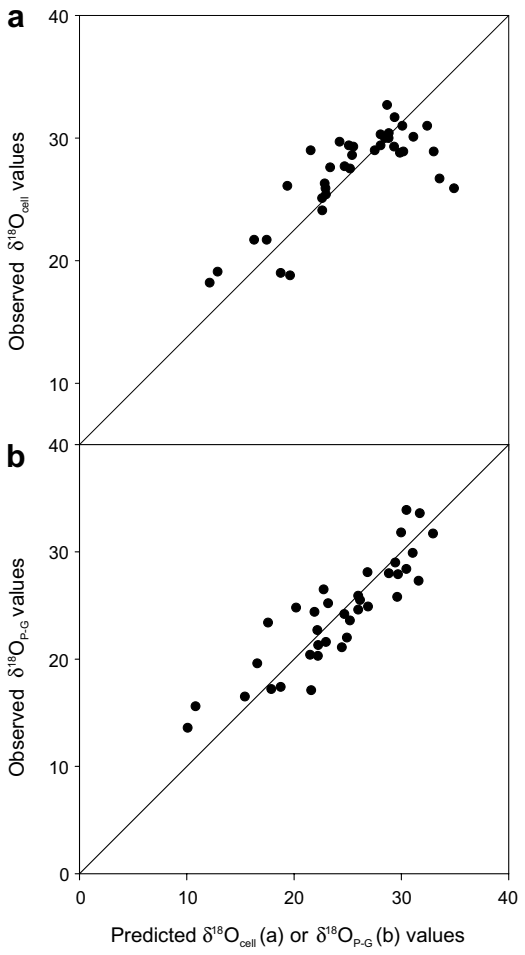


Fig. 4. Multiple linear regression observed cellulose (a) and phenylglucosazone (b) $\delta^{18}O$ values versus that predicted by a best-fit multiple linear regression based on $\delta^{18}O$ values of stem water and daytime growing season relative humidity. Solid line represents a one to one relationship.

plied by the $\delta^{18}O$ value of stem water, by using basic principles and a generalized tree-ring cellulose labeling model? The isotopic composition of heterotrophically synthesized cellulose in tree trunks and stems is given by

$$\delta^{18}O_{cell} = [F \cdot ((1 + \Delta_h \cdot 10^{-3}) \cdot \delta^{18}O_{sw} + \Delta_h)] + [(1 - F) \cdot ((1 + \Delta_a \cdot 10^{-3}) \cdot \delta^{18}O_{lw} + \Delta_a)] \quad (11)$$

in which $\delta^{18}O_{lw}$ is the $\delta^{18}O$ value of leaf water and $F$, $\Delta_h$ and $\Delta_a$ are: the proportion of oxygen that exchanges with stem water during heterotrophic cellulose synthesis, the biochemical mediated isotopic enrichment between the oxygen of cellulose or derivatives in relation to water at the site of synthesis during heterotrophic synthesis and the autotrophic oxygen isotopic enrichment of cellulose or derivative relative to the leaf water at the site of synthesis, respectively. The current model (Roden et al., 2000), however, simplifies the above equation to

$$\delta^{18}O_{cell} = [F \cdot (\delta^{18}O_{sw} + \Delta_h)] + [(1 - F) \cdot (\delta^{18}O_{lw} + \Delta_a)], \quad (12)$$

since $\Delta_h \cdot 10^{-3}$ or $\Delta_a \cdot 10^{-3}$ are such small quantities. According to the constant fractionation model the two enrichment factors above ($\Delta_h$ and $\Delta_a$) are assumed to be identical to 27‰, and they do not change with the position of the oxygen in the cellulose molecule or with changes in the isotopic composition of the source water.

The $\delta^{18}O$ value of leaf water ($\delta^{18}O_{lw}$) is determined by the gradient between the transpiration flux carrying isotopically un-enriched stem water and the back diffusion of isotopically enriched water from the evaporative surfaces internal to the leaf. The ratio of these two fluxes is described by the Peclet number

$$\rho = \frac{LE}{CD} \quad (13)$$

in which $L$ is the effective length of the diffusive pathway, $E$ is the transpiration rate of water, $D$ is the diffusivity of $H_2{}^{18}O$ in water and $C$ is the molar density of water (Farquhar and Lloyd, 1993). The $\delta^{18}O$ value of leaf water integrated over this diffusive and convective movement of water is described by

$$\delta^{18}O_{lw} = \alpha \cdot \delta^{18}\,O_{ew} + (1 - \alpha) \cdot \delta^{18}O_{sw}, \quad (14)$$

in which $\alpha = \frac{1-e^{-\rho}}{\rho}$, and $\delta^{18}O_{ew}$ is the $\delta^{18}O$ value of water associated with the internal evaporative surfaces of the leaf, given by

$$\delta^{18}O_{ew} = \delta^{18}O_{sw} + \varepsilon_{vq} + \varepsilon_{vk} + RH \cdot (\delta^{18}O_a - \varepsilon_{vk} - \delta^{18}O_{sw}), \quad (15)$$

$\delta^{18}O_a$, $\varepsilon_{vq}$ and $\varepsilon_{vk}$ are the $\delta^{18}O$ value of atmospheric vapor, equilibrium isotopic fractionation factor between vapor and liquid water and the kinetic isotopic fractionation for the diffusivity of water, respectively (Dongmann et al., 1974). When atmospheric vapor is at equilibrium with the source (stem) water then the above equation is simplified to

$$\delta^{18}O_{ew} = \delta^{18}O_{sw} + [(\varepsilon_{vq} + \varepsilon_{vk}) \cdot (1 - RH)]. \quad (16)$$

Merging Eqs. (12), (14) and (16) together:

$$\delta^{18}O_{cell} = [F \cdot \Delta_h + (1 - F) \cdot \Delta_a + \alpha \cdot (1 - F) \cdot (\varepsilon_{vq} + \varepsilon_{vk})] - [\alpha \cdot (1 - F) \cdot (\varepsilon_{vq} + \varepsilon_{vk})] \cdot RH + [1] \cdot \delta^{18}O_{sw}. \quad (17)$$

The bracketed terms above should approximate the constants of the best-fit linear equation:

$$A_0 = [F \cdot \Delta_h + (1 - F) \cdot \Delta_a + \alpha \cdot (1 - F) \cdot (\varepsilon_{vq} + \varepsilon_{vk})], \quad (18)$$

the factor which is constant;

$$A_1 = -[\alpha \cdot (1 - F) \cdot (\varepsilon_{vq} + \varepsilon_{vk})], \quad (19)$$

the factor which is multiplied by the relative humidity; and

$$A_2 = 1, \quad (20)$$

the factor which multiplies the $\delta^{18}O$ value of stem water. When these expressions are evaluated with the reasonably assumed values for the parameters $\alpha$, $\varepsilon_{vq}$, $\varepsilon_{vk}$ and $F$ (Table 3), however, the values of $A_0$, $A_1$ and $A_2$ are very different from those reported for the best linear fit between $\delta^{18}O_{cell}$ and the independent variables $\delta^{18}O_{sw}$ and RH

Table 3
Comparisons of the coefficients $A_0$, $A_1$ and $A_2$ of a best-fit linear equation predicting $\delta^{18}O_{cell}$ or $\delta^{18}O_{P-G}$ as a function of $\delta^{18}O_{sw}$ and RH with coefficients derived by two different types of models: one assuming fixed isotopic fractionation and the other assuming variable isotopic fractionation

| | Best-fit multiple linear regression | | Model fixed fractionation | | Model variable fractionation | |
|---|---|---|---|---|---|---|
| | Cellulose | P–G | Cellulose | P–G | Cellulose | P–G |
| $F_{obs}$ | n.a. | n.a. | 0.402[a] | 0.337[a] | 0.402[a] | 0.337[a] |
| $\beta$ | n.a.. | n.a. | n.a. | n.a. | 0.110[b] | 0.076[b] |
| $\Delta_a$ | n.a. | n.a. | 27.0[c] | 27.0[c] | 28.3[b] | 22.5[d] |
| $\varepsilon_{vq}$ | n.a. | n.a. | 9.3[e] | 9.3[e] | 9.3[e] | 9.3[e] |
| $\varepsilon_{vk}$ | n.a. | n.a. | 28.5[e] | 28.5[e] | 28.5[e] | 28.5[e] |
| $F_{act}$ | n.a. | n.a. | 0.402 | 0.337 | 0.451 | 0.365 |
| $\rho$ | n.a. | n.a. | 0.50[f] | 0.50[f] | 0.50[f] | 0.50[f] |
| $A_0$ | 41.72 | 43.87 | 44.85 | 46.80 | 41.72 | 43.87 |
| $A_1$ | −10.63 | −17.47 | −17.85 | −19.80 | −14.57 | −17.53 |
| $A_2$ | 0.948 | 1.076 | 1.000 | 1.000 | 0.890 | 0.924 |
| $\Delta_h$ | n.a. | n.a. | 27.0 | 27.0 | 25.6 | 32.9 |
| $\Delta_{average}$ | n.a. | n.a. | 27.0 | 27.0 | 27.1 | 26.3 |
| Residual | 2.20 | 2.05 | **2.99** | **2.94** | **2.89** | 2.32 |

Also shown are the values of the parameters used in the calculation of the model equations and the average residuals between equations and observations. Bold values in the residuals row indicate significantly higher values than those from the best-fit equation ($P < 0.05$, paired $T$ test).

[a] Values derived from cellulose and derivative grown heterotrophically from wheat seeds having starch as the primary substrate (Sternberg et al., 2006).

[b] Based on the average of aquaria experiments (Sauer et al., 2001) and *Ricinus communis* germination experiments (Sternberg et al., 2006).

[c] The commonly used fixed average oxygen isotope fractionation relative to available water during cellulose synthesis (Roden et al., 2000).

[d] Fractionation calculated for phenylglucosazone derived from cellulose synthesized in the *Ricinus communis* germination experiment (Sternberg et al., 2006).

[e] Commonly used equilibrium and kinetic fractionation factors in standard tree-ring models (Roden et al., 2000). We assume 25 °C for the equilibrium fractionation factor $\varepsilon_{vq}$.

[f] Average peclet number for conifer and evergreens in a botanical garden survey of several species (Wang et al., 1998).

(Table 3). The residuals between observed $\delta^{18}O_{cell}$ values versus that predicted by the above model were significantly greater than those generated by the best-fit multiple linear regression (Table 3).

We can use the same set of equations above with different values of $F$ to model $\delta^{18}O_{P-G}$ values (Table 3). As in the above equations, we assume that the isotopic fractionations during exchange between oxygen attached to carbon 3–6 are constant and 27‰ for both heterotrophic and autotrophic cellulose synthesis. The coefficients $A_0$, $A_1$ and $A_2$ for the phenylglucosazone derivative model are also considerably different than those calculated by the best-fit multiple linear regression (Table 3). The residuals between the observed $\delta^{18}O_{P-G}$ and predicted by this model are also significantly higher than those generated by the best-fit multiple linear regression (Table 3).

#### 4.4.2. The variable fractionation model

This study indicates that the oxygen isotopic fractionation during cellulose synthesis decreases with an increase in the isotopic composition of water (Figs. 2a and 3) and we previously speculated that this may be caused by the presence of intracellular metabolic water. If this is the case, what has been considered as biochemical fractionations is really the net effect of the isotopic ratios of metabolic water and biochemical fractionations associated with cellulose synthesis. The major implication of the changing net-fractionation with a change in the $\delta^{18}O$ value of source water is that the previous tissue cultures, seed germination, aquaria and hydroponic experiments where cellulose is synthesized either autotrophically or heterotrophically underestimated the proportion of oxygen in the cellulose molecule that exchanges with water during cellulose synthesis ($F$). For example, in an aquarium experiment Sauer et al. (2001) carefully monitored the isotopic composition of aquaria water and observed an $F$ for cellulose synthesized autotrophically of 0.88. This is lower than the expected value ≈1, since it is considered that water is the only source of oxygen labeling during cellulose synthesis. Sternberg et al. (2006) in a seed germination experiment, where the major source of carbon for cellulose synthesis was oxygen poor lipid stored in *Ricinus communis* seeds, observed an $F$ of 0.905, when again it should have been ≈1, since the oxygen for the carbohydrate synthesis must have been solely provided by water. The high correlation coefficient of 1 for both of these experiments eliminates the possibility that these slopes are lower than expected because of analytical errors. Consider the equation describing autotrophic cellulose synthesis

$$\delta_{ac} = F\delta_w + \Delta_a. \quad (21)$$

In which $\delta_{ac}$ and $\delta_w$ are the $\delta^{18}O$ values of autotrophic synthesized cellulose and the available water, respectively. In the above case it is expected that $F$ should be very close to 1 (=1.027) but investigators usually observe a slope less than 1 ranging from 0.48 to 0.88 (Cooper and DeNiro, 1989; Yakir and DeNiro, 1990; Sauer et al., 2001). However, if we consider that the autotrophic fractionation

decreases linearly by a factor $\beta$ with an increase in the $\delta^{18}O$ value of water, then:

$$\delta_{ac} = F\delta_w + (\Delta_{a,0} - \beta \cdot \delta_w). \quad (22)$$

In which $\Delta_{a,0}$ is the autotrophic oxygen isotopic fractionation during cellulose synthesis in water with a $\delta^{18}O$ value of 0‰. This equation will simplify to

$$\delta_{ac} = (F - \beta) \cdot \delta_w + \Delta_{a,0}, \quad (23)$$

which is the relationship observed in several of the above controlled experiments: $F_{obs} = (F - \beta)$ being less than $F$. Similarly, if we assume that the heterotrophic fractionation also decreases with an increase in the $\delta^{18}O$ value of water during synthesis as in the autotrophic fractionation, then the observed amount of exchangeable oxygen during heterotrophic cellulose synthesis would equal to $F(1 - \beta)$, which is less than the expected $F$.

Given the above assumption about changes in the net isotopic fractionation factor with an increase in the $\delta^{18}O$ value of water, the calculations of coefficients $A_0$, $A_1$ and $A_2$ are modified to

$$A_0 = \left(\frac{F_{obs}}{1-\beta}\right) \cdot \Delta_{h0} + \left(1 - \frac{F_{obs}}{1-\beta}\right) \cdot \Delta_{a0} + [\alpha \cdot (1 - F_{obs} - \beta) \cdot (\varepsilon_{vq} + \varepsilon_{vk})], \quad (24)$$

$$A_1 = -\alpha \cdot (1 - F_{obs} - \beta) \cdot (\varepsilon_{vq} + \varepsilon_{vk}) \text{ and} \quad (25)$$

$$A_2 = 1 - \beta. \quad (26)$$

In which $\Delta_{h0}$ and $\Delta_{a0}$ are the heterotrophic and autotrophic isotopic fractionation during cellulose synthesis in water having a $\delta^{18}O$ value of 0‰; here forthwith written as the fractionation at 0. To estimate the $\beta$ factor ($\beta_{cell}$) and $\Delta_{a0}$ for cellulose, we consider the case where all the oxygen from cellulose was derived from water as in the *R. communis* experiment. The observed $F_{cell}$ was 0.905 with a fractionation at 0 of 28.4‰ for cellulose. The observed slope is 0.095 short of a slope of 1 which is the expected value of $F_{cell}$, therefore $\beta_{cell} = 0.095$. We note that a more accurate estimate of the deviation would be to compare the observed slope with the expected $1+\Delta_a \cdot 10^{-3}$. However, to keep this model simple and in line with the simplifications of the standard model, we will compare the observed slope with 1. Likewise, Sauer et al. (2001) in the aquaria experiment where all the oxygen of the cellulose were derived from water observed a slope of 0.88 with a fractionation at 0 of 28.3‰, making the value of $\beta_{cell}$ of 0.12. We will adopt the average values of the two studies above for $\beta_{cell}$ of 0.11 and the average fractionation at 0 of 28.3‰. For the phenylglucosazone $\beta$ factor ($\beta_{P-G}$) and $\Delta_{a0}$ we used data from the previous *R. communis* study (Sternberg et al., 2006) showing that the slope of the relationship between $\delta^{18}O_{P-G}$ and the $\delta^{18}O$ value of the available water was 0.924 when it should have been 1, therefore $\beta_{P-G}$ has a value of 0.076. We used the fractionation of 22.5‰ as the autotrophic isotopic fractionation based on the above study (Sternberg et al., 2006). In the variable fractionation model we assume that $A_0$ is the same as that generated by the multiple linear regression and given the respective adjusted values of $F$ and $\Delta_{a0}$, we solve for the heterotrophic fractionation ($\Delta_{h0}$). Therefore, we allow for differences in fractionation depending on the heterotrophic versus autotrophic synthesis.

For both cellulose and phenylglucosazone, we observed that the weighed average of the autotrophic and heterotrophic isotopic fractionation values were 27.1‰ and 26.3‰, respectively, which are similar to the reported average 27‰ isotopic fractionation during carbohydrate synthesis. The fractionation factors for heterotrophic and autotrophic synthesis however differed (Table 3). Using the above parameters we see that the coefficients for the relationship between $\delta^{18}O_{cell}$ versus $\delta^{18}O_{sw}$ and RH are still considerably different than those from the best-fit linear regression (Table 3). The residuals are also significantly greater than those generated by the best-fit model (Table 3). For the phenylglucosazone derivative, however, we observed a significant improvement in the likeness of the coefficients to those of the best-fit equation and no significant difference was observed between the residuals generated from the modeled $\delta^{18}O_{P-G}$ and those from the best-fit equation (Table 3).

## 5. CONCLUSIONS

We have shown here that the isotopic composition of the oxygen attached to the second carbon of the cellulose–glucose moieties can introduce isotopic "noise" during the recording of environmental factors by cellulose. However, $\delta^{18}O_{C-2}$ values may be less noisy when measured across a chronological sequence of tree rings within a single individual and the application of $\delta^{18}O_{C-2}$ measurement to separate leaf signals from trunk signals may still be possible. By deriving cellulose into phenylglucosazone, we improved the relationship between the remaining oxygen in the cellulose molecule and source water and relative humidity. We have also shown that current mechanistic models must incorporate the observation that oxygen isotopic fractionations for oxygen attached to carbons 3–6 during cellulose synthesis changes in relation to the isotopic composition of the available water source, and that these fractionations can differ between autotrophic and heterotrophic synthesis. By incorporating these changes we improved the model predictions to be undistinguishable from the best-fit linear regression between $\delta^{18}O_{P-G}$ versus $\delta^{18}O_{sw}$ and RH.

Our observation of a changing oxygen isotopic fractionation during cellulose synthesis has an impact on the interpretation of previous and current studies of tree-ring oxygen isotope ratios. This observation raises the question of whether changes in the oxygen isotope ratio along a tree-ring chronological sequence are brought about by either environmental effects (source water or RH) or by metabolic effects. Derivation of tree-ring cellulose to phenylglucosazone may give us the tool to determine the nature of the isotopic signal (metabolic versus environmental) since much of the isotopic noise caused by metabolism is observed in the oxygen attached to the second carbon of the glucose moieties. However, routine use of this method on tree-ring chronological sequences remains a challenge because the "wet chemistry" is involved and the amount of cellulose needed for the derivation is large (300 mg) compared to that needed for oxygen isotope analysis of tree-ring cellulose

(~1 mg). Therefore, isotopic analysis of intra-molecular oxygen in cellulose, presently, can only be used to check on the nature of major shifts in the oxygen isotope ratios of cellulose from tree-ring sequences.

We have not solved the problem of disentangling the cellulose oxygen isotopic signals of the leaf from those of the trunk water, which was the original intent of this research. We note however that, if it is known that the RH did not shift to values below 33% during a chronological sequence, the correlation between $\delta^{18}O_{sw}$ and $\delta^{18}O_{P-G}$ is high ($r = 0.91$). In which case, the derivation of cellulose to phenylglucosazone followed by oxygen isotopic analysis can be used to determine major shifts in plant available water brought about by either change in climate or hydrological processes. Since the $\delta^{18}O$ values of tree-ring cellulose along a chronological sequence are often a mixture of the isotope ratios of stored and current photosynthates, it is important to calibrate this method along a chronological sequence of tree-ring cellulose for trees at a site where rainfall oxygen isotope ratios have been consistently recorded for a period of time.

## ACKNOWLEDGMENTS

We thank Lisa Welp (Caltech Environmental Science and Engineering) for the donations of stem samples from Alaska and Siberia. This research was supported by NSF Grant OPP 0323877 to H.J. and OPP 0322364, DBI 0420533 to L.S.

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*Associate editor:* Jay A. Brandes