Oxygen stable isotope ratios of tree-ring cellulose: the next phase of understanding

Leonel da Silveira Lobo O'Reilly Sternberg
Department of Biology, University of Miami, Coral Gables, FL 33124, USA

Summary

Analysis of the oxygen isotope ratio of tree-ring cellulose is a valuable tool that can be used as a paleoclimate proxy. Our ability to use this tool has gone through different phases. The first began in the 1970s with the demonstration of empirical relationships between the oxygen isotope ratio of tree-ring cellulose and climate. These empirical relationships, however, did not provide us with the confidence that they are robust through time, across taxa and across geographical locations. The second phase began with a rudimentary understanding of the physiological and biochemical mechanisms responsible for the oxygen isotope ratios of cellulose, which is necessary to increase the power of this tool. This phase culminated in a mechanistic tree-ring model integrating concepts of physiology and biochemistry in a whole-plant system. This model made several assumptions about leaf water isotopic enrichment and biochemistry which, in the nascent third phase, are now being challenged, with surprising results. These third-phase results suggest that, contrary to the model assumption, leaf temperature across a large latitudinal gradient is remarkably constant and does not follow ambient temperature. Recent findings also indicate that the biochemistry responsible for the incorporation of the cellulose oxygen isotopic signature is not as simple as has been assumed. Interestingly, the results of these challenges have strengthened the tree-ring model. There are several other assumptions that can be investigated which will improve the utility of the tree-ring model.

Introduction

Oxygen stable isotope ratios of tree-ring biomass can be used as a proxy for climate during tree growth. The basic principle of this tool is the observation that the oxygen stable isotope ratio of precipitation (expressed here as the $\delta^{18}O$ value) is related to temperature and at least part of this isotopic climate signal is passed on to the isotope ratios of plant biomass. The ongoing development of this tool can be roughly divided into three phases. The first phase involved showing empirical relationships between $\delta^{18}O$ values of plant biomass and climate. Libby et al. (1976) produced one of the earliest works...
that correlated the oxygen isotope ratios of tree-rings in two German oak trees (Quercus petraea) with temperature. This work was followed by several other studies that established empirical relationships between oxygen isotope ratios of tree-ring cellulose or wood and measured climatic parameters such as temperature, relative humidity (RH) and precipitation (Burk & Stuiver, 1981; Ramesh et al., 1986; Saurer et al., 1997; Robertson et al., 2001; Rebetz et al., 2003). Empirical relationships between the oxygen isotope ratios of plant biomass and climate have been used by some to project back in time to before climate records were kept (Masson-Delmotte et al., 2005; Ballantyne et al., 2006; Treydte et al., 2006). The above studies, which relied on empirical relationships without an understanding of the underlying physiological and biochemical mechanisms, suffered from the weakness of not knowing whether these relationships are robust through time, across taxa and across geographical locations. This brings us to the second phase: the construction of a mechanistic model to explain the incorporation of $^{18}$O into plant biomass. This second phase culminated in the Roden et al. (2000) tree-ring model which integrated our understanding of oxygen isotope fractionations associated with water passing through the plant and the biochemical process of cellulose synthesis. This framework model of tree-ring cellulose allows us to better understand how the ecophysiological and biochemical behavior of trees affects the oxygen isotope ratios of tree-ring cellulose. Several assumptions regarding leaf temperature, the isotopic composition of atmospheric vapor and biochemical fractionations, among others, were made in establishing this model. The nascent third phase of our understanding involves testing and determining the impact of the above assumptions on the $\delta^{18}$O values of tree-ring cellulose. In this review, I will discuss the development of the basic tree-ring model (second phase) and some recent developments which introduce us to the third phase of understanding of tree-ring oxygen isotope ratios. Finally, I will discuss future directions to improve our interpretation of stable isotopes in tree-ring cellulose.

Development of a mechanistic tree-ring isotope model

The development of a mechanistic model predicting the oxygen isotope ratios of tree-ring cellulose based on climate was the product of two parallel lines of study: elucidation of (1) the changes in the oxygen isotope ratio of water as it moves through the plant and (2) the changes in the isotope ratio of oxygen as it becomes incorporated in the cellulose molecule through biochemical effects. Studies on the changes of the isotope ratio of water as it moves through the plant focused on the only plant organ that contains water with a different oxygen isotopic composition from that taken up by the plant roots: the leaf. Interest in leaf water was stimulated not only by Epstein et al.’s seminal work showing that the oxygen isotope ratio of cellulose is related to that of water (Epstein et al., 1977), but also by the observations that molecular oxygen with the isotopic composition of leaf water is released during photosynthesis (Guy et al., 1993) and that ambient CO$_2$ absorbed by the leaf isotopically equilibrates with leaf water and is partly released back to the atmosphere (Farquhar & Lloyd, 1993). The isotopic labeling of these two gases provides an important geochemical tool with which to elucidate the global oxygen and carbon cycle (Dole et al., 1954; Francey & Tans, 1987; Farquhar et al., 1993; Bender et al., 1994).

Oxygen isotope ratios of leaf water

The isotopic composition of the evaporative pool of leaf water undergoing transpiration ($\delta^{18}$O$_L$) is given by the Craig–Gordon equation (Craig & Gordon, 1965):

$$\delta^{18}O_E = \delta^{18}O_S + \varepsilon^* + \varepsilon_k + \varepsilon_{e} \left( \delta^{18}O_A - \varepsilon_k - \delta^{18}O_S \right) \quad \text{Eqn 1}$$

The isotopic composition of the evaporating pool of water in the leaf is affected by the ratio of ambient vapor pressure to that inside the leaf ($e/e_0$), the $\delta^{18}$O value of atmospheric vapor ($\delta^{18}O_A$) and the equilibrium and kinetic isotopic effects ($\varepsilon^*$ and $\varepsilon_k$, respectively) in addition to the isotopic composition of stem water ($\delta^{18}O_S$).

Several investigators measured the isotopic composition of leaf water ($\delta^{18}O_L$) under different RHs and, although the isotopic enrichment trends were consistent with Eqn 1, leaf water was often not as enriched as predicted by the Craig–Gordon model (see Cuntz et al., 2007 for a review). This discrepancy was explained by the presence of a nonenriched leaf water compartment (having a similar $\delta^{18}$O value to that of stem water) as well as the presence of an enriched leaf water compartment with a $\delta^{18}$O value similar to that predicted by Eqn 1. Bulk leaf water isotopic composition was, therefore, proposed to be the weighted average of the isotopic compositions of these two water compartments (Planagan & Ehleringer, 1991).

One of the weaknesses of the above compartmentation hypothesis is the assumption that water compartments are discrete and of a constant size. This assumption cannot fully explain the pattern of leaf water enrichment. For example, given the same vapor pressure deficit, water in a leaf that has low transpiration rates becomes isotopically enriched compared with that of a leaf that has high transpiration (Cuntz et al., 2007). Interestingly, a common conversational error is to state the opposite: ‘leaf water is isotopically enriched because of high transpiration’. This pattern is best predicted by the model proposed by Farquhar & Lloyd (1993) where the isotopic composition of bulk leaf water is determined by the balance between two water fluxes in opposite directions. In one direction, from the apoplast towards the leaf vein, there is a diffusional flux of the isotopically enriched water having the isotope ratio predicted by Eqn 1 and in the opposite direction there is the transpiration-driven convective flux of isotopically nonenriched
stem water towards the apoplast. The net effect of convective and diffusive flow is known as the Péclét effect ($\rho$) which is dimensionless and given by the following equation:

$$\rho = (TL)/(CD) \quad \text{Eqn 2}$$

($C$, the molar concentration of water ($5.56 \times 10^4$ mol m$^{-3}$); $D$, the diffusivity of $H_2^{18}O$ in water ($2.66 \times 10^{-9}$ m$^2$ s$^{-1}$); $T$ (mol m$^{-2}$ s$^{-1}$), the rate of transpiration; $L$ (m), the effective mixing length in the leaf.) The effective mixing length is dependent on the tortuosity of the diffusive pathway as well as the physical distance from vein to apoplast (Barbour & Farquhar, 2004). The bulk leaf isotopic composition is predicted by the following equation:

$$\delta_e = [(1 - \alpha)\delta_p] + \alpha\delta_\rho$$  \hspace{1cm} \text{Eqn 3}$$

where $\alpha$ is given by $(1 - e^{-\rho})/\rho$. Leaf water can be thought of as being determined by the fraction of isotopically nonenriched water $(1 - \alpha)$ and the fraction $(\alpha)$ of enriched water. These fractions, however, reflect a continuous gradient and not a discrete compartment; this gradient is also dynamic and subject to change depending on the transpiration rate of the leaf. Note that the greater the transpiration ($T$), the greater the Péclét effect (Eqn 2), which reduces the contribution of enriched water $(\alpha)$. The effect of transpiration in lowering the $\delta^{18}O$ values of bulk leaf water discussed above can, therefore, be explained by this equation.

### Biochemical effects during cellulose synthesis

The $\delta^{18}O$ value of cellulose ($\delta^{18}OC$) is $27\%o$ ($\pm 4\%o$) higher than that of the water ($\delta^{18}OW$) present during its synthesis (Epstein et al., 1977; Denirno & Epstein, 1981). This constant relationship was first hypothesized to be caused by the contribution of one oxygen atom from water and two from $CO_2$ equilibrated with ambient water towards carbohydrate synthesis. As the $\delta^{18}O$ value of $CO_2$ equilibrated with water is $-40.0\%o$ higher than that of the water it equilibrated with, the above process can be summarized by:

$$\delta^{18}OC = (\delta^{18}OW/3) + [2/3(\delta^{18}OW + 40\%o)] \quad \text{Eqn 4}$$

which simplifies to:

$$\delta^{18}OC = \delta^{18}OW + 26.7\%o$$  \hspace{1cm} \text{Eqn 5}$$

However, if only pre-photosynthetic equilibration of $CO_2$ with water determines the $\delta^{18}O$ value of cellulose, then one would expect a temperature effect on the oxygen isotope ratios of cellulose. This is because there is a significant and easily detected temperature effect on the fractionation factor for the $CO_2$/water equilibration of $0.2\%o$ °C$^{-1}$. DeNirno & Epstein (1981) grew aquatic plants at different temperatures ranging from 25 to 35°C and found no significant differences in the fractionation between cellulose and water as a function of temperature. These results indicate that post-photosynthetic exchange reactions occurring between carbohydrate intermediates and water during cellulose synthesis play an important part in determining the $\delta^{18}O$ values of cellulose.

Sternberg & DeNirno (1983) hypothesized that post-photosynthetic exchange reactions during cellulose synthesis occur during carbonyl hydration:

$$\begin{align*}
\text{C} + \text{H}_2\text{O} & \rightleftharpoons \text{OH} \quad \text{HO} \quad \text{C} \quad \text{OH}
\end{align*} \quad \text{Eqn 6}$$

They tested the magnitude of the oxygen isotope fractionation between carbonyl oxygen and water for the carbonyl hydration reaction in acetone, as a model molecule. They found a fractionation factor of $-27\%o$, which is similar to the fractionation factor between cellulose and the available water during its synthesis.

Firm evidence for the importance of post-photosynthetic reactions in the determination of the $\delta^{18}O$ values of cellulose was provided by experiments on heterotrophic cellulose synthesis from tissue culture, Lemma gibba cultures and seed germination (Sternberg et al., 1986; YakIR & DeNirno, 1990; Luo & Sternberg, 1992; Sternberg et al., 2006). In these experiments it was observed that for a carbohydrate substrate, such as sucrose or starch, approx. 40% of the oxygen destined for cellulose became labeled by the water of the culture media or the water available for seed germination. The exchange reaction is thought to occur through the carbonyl hydration reaction during the futile cycle in which fructose 1,6-Phosphate recycles through triose phosphates (Hill et al., 1995). The above post-photosynthetic exchange of carbohydrate oxygen with water during heterotrophic cellulose synthesis can be summarized as:

$$\delta^{18}OC = [\phi(\delta^{18}OW + \Delta)] + [(1 - \phi)(\delta^{18}OSUB)] \quad \text{Eqn 7}$$

($\phi$, the proportion of oxygen that exchanged with water during cellulose synthesis; $\Delta$, the average fractionation for all oxygen that exchanged with water during cellulose synthesis; $\delta^{18}OSUB$, the average $\delta^{18}O$ value of the oxygen in the original substrate (sucrose or starch) that did not exchange with water (Sternberg et al., 1986).)

### Tree-ring isotope model

The tree-ring oxygen isotope model represents a synthesis of knowledge concerning the physiological processes controlling the oxygen isotope composition of leaf water with that concerning biochemical isotopic effects during cellulose synthesis (Roden et al., 2000; Barbour et al., 2004; Fig. 1). It is well known that leaf water becomes isotopically enriched during transpiration, with the Péclét effect being important in
determining the isotopic composition of bulk leaf water. One question that is critical to this model is whether the $\delta^{18}O$ value of sucrose synthesized in the leaf reflects the isotopic composition of a particular leaf water fraction or that of bulk leaf water. That it reflects bulk leaf water (as predicted by Eqn 3) was elegantly shown by the isotopic analysis of sucrose exudates and leaf water from castor bean (Ricinus communis) leaves (Barbour et al., 2000). In another study it was also shown that there are no isotopic effects during sucrose translocation (Gessler et al., 2007).

The tree-ring isotope model calculates the leaf water isotopic composition based on the isotopic composition of stem water and atmospheric vapor, vapor pressure ratios and the Péclet number of the leaf (Eqns 1, 2 and 3). The $\delta^{18}O$ value of sucrose synthesized in the leaf is then calculated by:

$$\delta^{18} O_{\text{SUB}} = \delta^{18} O_L + 27\%$$  \hspace{1cm} \text{Eqn 8}$$

Sucrose is translocated and, as it is allocated to cellulose synthesis in the trunk (heterotrophic cellulose synthesis), an average of 0.42 of the oxygen destined for the cellulose molecule exchanges with stem water. The $\delta^{18}O$ value of cellulose can be calculated using Eqn 7, with $\phi$ as the above proportion of 0.42 (Roden et al., 2000). Throughout this model the oxygen isotope fractionation for the exchangeable oxygen in carbohydrate substrates destined for cellulose synthesis is assumed to average 27% relative to the $\delta^{18}O$ value of the water present during cellulose synthesis. It is also usually assumed that the atmospheric vapor is at equilibrium with the plant available water (stem water) and that leaf temperature follows the ambient temperature. The latter assumption allows
us to substitute RH for the vapor pressure ratio ($\varepsilon / \varepsilon$) in Eqn 1. With these assumptions, this model has only two major unknowns: the $\delta^{18}O$ value of the water available to the plant (stem water) and the RH. Although this model represents a leap in our understanding of tree-ring cellulose, it cannot be used in paleoclimate reconstruction unless either the $\delta^{18}O$ value of plant available water or the RH is known. One method for solving for the two unknowns was proposed by Jahren & Sternberg (2003) using two simultaneous equations each expressing the $\delta^{18}O$ and the $\delta^D$ values of the nonexchangeable hydrogen of cellulose.

The tree-ring isotope model gives expected results in some cases (Roden & Ehleringer, 2000; Roden et al., 2005) and, when inconsistencies are found, they are explained on the basis of different water sources or the variation in the biochemical processes (Waterhouse et al., 2002; Roden et al., 2005). In one of the studies cited above (Waterhouse et al., 2002), which used a best fit approach, the empirical values derived for the biochemical fractionation factor between cellulose and water (30‰) and $\Phi$ (0.46) were different from the previously observed averages of 27‰ and 0.42, respectively. Many of the above inconsistencies can be explained by further investigating the underlying assumptions of the tree-ring model; the third phase of understanding the tree-ring model.

**Recent developments**

**Testing assumptions regarding leaf water isotopic enrichment**

Two of the principal assumptions regarding the isotope ratios of leaf water are: (1) that the integrated $\delta^{18}O$ value of atmospheric vapor is the same as that of vapor in isotopic equilibrium with plant water or annual average precipitation and (2) that the average leaf temperature during carbohydrate synthesis is the same as ambient temperature. The assumption about the $\delta^{18}O$ values of atmospheric vapor is adopted, in part, out of necessity, because there are few measurements of the isotopic composition of atmospheric vapor, particularly on an integrative basis. To test this assumption one needs to collect atmospheric vapor for a prolonged period at the same time as monitoring the long-term isotopic composition of precipitation. Collecting atmospheric vapor requires the use of cold traps, flow meters and pumps over a sustained period of time. In addition, collection of vapor is subject to fractionation in the case of inefficient cold traps. This is a critical assumption in that small errors in the estimate of the $\delta^{18}O$ value of atmospheric vapor can lead to large errors in tree-ring data. A 2‰ error in the estimate of the $\delta^{18}O$ value of atmospheric vapor as it propagates to the $\delta^{18}O$ values of tree-ring cellulose can be equivalent to up to 66% of the observed range in tree-ring signals (Helliker & Griffiths, 2007). Epiphytes, which have their water at equilibrium with atmospheric vapor, may provide long-term proxies of the $\delta^{18}O$ value of atmospheric vapor (Helliker & Griffiths, 2007). Analysis of the organic matter in Tillandsia usneoides (Spanish moss) in Miami spanning 127 yr and modeling approaches indicate the isotopic equilibrium of atmospheric vapor with year-round precipitation (Helliker & Griffiths, 2007). Tillandsia usneoides, however, only grows in selected locations with relatively high humidity and it is possible that in dry locations the isotopic composition of atmospheric vapor does not reflect isotopic equilibrium with year-round precipitation. However, note that the impact of the error in the estimate of the $\delta^{18}O$ value of atmospheric vapor diminishes as vapor pressure deficit increases (Eqn 1).

The assumption that leaf temperature is the same as ambient temperature allows one to use ambient RH for the vapor pressure ratio term in Eqn 1. However, a recent survey of tree-ring data encompassing a latitudinal range of 50° indicated a surprising deviation between leaf temperature and growing season temperature, particularly at higher latitudes (Helliker & Richter, 2008). Helliker & Richter (2008), in a survey of tree-ring cellulose, observed that the $\delta^{18}O$ values of tree-ring cellulose deviated considerably from that predicted by the tree-ring model when it is assumed that leaf and growing season ambient temperature are the same. This deviation was particularly large at higher latitudes with lower mean annual temperatures (MATs). However, the observed values can be seen to be in closer agreement with modeled values if one considers that leaf temperature is higher than the ambient growing season temperature and MAT. Helliker & Richter (2008) observed a remarkably constant leaf temperature of 21.4 ± 2.2°C for all latitudes when they reverse-solved the tree-ring model for temperature (Fig. 2), indicating that at higher latitudes the leaf temperature of actively photosynthesizing trees could be considerably higher than the growing season temperature. Therefore, the assumption that leaf temperature is equal to ambient temperature during photosynthesis may not be applicable. There are several ways in which plants can regulate leaf temperature, such as through cooling produced by transpiration or heating produced as a result of the canopy structure (Helliker & Richter, 2008; Woodward, 2008). However, further corroborative evidence is needed regarding the constancy of leaf temperature because there are alternate explanations for the isotopic pattern observed by Helliker & Richter (2008). Richter et al. (2008), for example, explained the observed isotopic pattern on the basis of different effects of RH or the isotope ratios of vapor at different latitudes. In addition, the assumption that the biochemical oxygen isotope fractionation during cellulose synthesis is constant may not be valid, as a pattern of diminishing fractionation with decrease in latitude has also been reported for aquatic plants (Sternberg et al., 2007). The near-constant leaf temperature, if shown to be real, allows us to discard the assumption that leaf temperature is the same as ambient temperature and replace it with the constant leaf temperature of 21.4°C as observed by Helliker & Richter (2008); at least for biomes having a MAT < 15°C (Woodward, 2008). This would greatly simplify
the application of the tree-ring model. This finding should also have implications for the use of the $\delta^{18}O$ values of atmospheric O$_2$ and CO$_2$ to budget atmospheric oxygen and carbon dioxide. These budgets must take into account the fact that, if there is a constant leaf temperature, the leaf water value will be significantly different from that predicted if the leaf temperature is the same as the growing season temperature. In addition, they must take into account the fact that the isotopic equilibration of CO$_2$ and leaf water is determined by a fractionation factor at leaf temperature rather than ambient temperature.

Testing assumptions about biochemical fractionations during cellulose synthesis

The major assumption regarding the oxygen isotope ratios of cellulose is that the constant 27‰ fractionation between cellulose and water is caused by the exchange between carbonyl oxygens of carbohydrates and water during cellulose synthesis. However, Schmidt et al. (2001) suggested that the match between the average 27‰ cellulose fractionation and that of the carbonyl hydration reaction of acetone, as measured by Sternberg & DeNiro (1983), is coincidental. They proposed that the oxygen isotopic fractionation of cellulose relative to water is the average of several different fractionation effects occurring at different stages and in different oxygens of the glucose moiety during the synthesis of cellulose. Schmidt et al.'s conjecture was shown to be correct when Sternberg et al. (2003, 2006) developed a technique for deriving phenyl-glucosazone from stem cellulose and analyzing the isotope ratio of the oxygen attached to the second carbon of the cellulose glucose moiety ($\delta^{18}O_{C-2}$) separately from that of the oxygen attached to carbons 3, 4, 5 and 6. The isotopic fractionation relative to water for the oxygen attached to the second carbon ($51.0 \pm 5.0$‰) differs from the average for the oxygen attached to carbons 3, 4, 5 and 6 ($22.5 \pm 2.0$‰) for cellulose synthesized from oxygen-poor lipids (Sternberg et al., 2006). Nevertheless, the weighted average fractionation for the whole cellulose molecule was $28.2\% = (0.20 \times 51.0) + (0.80 \times 22.5)$, well within the range of reported fractionations for cellulose synthesis in the literature.

There is a greater exchange between the oxygen attached to the second carbon of the cellulose glucose moieties (70%)
compared with the other oxygens in the glucose moieties (33.6%) during heterotrophic cellulose synthesis (Sternberg et al., 2006). However, there are biochemical reactions particular to this oxygen which superimpose isotopic noise in the climate signal of the cellulose molecule (Sternberg et al., 2007). In a wide geographical survey of the $\delta^{18}$O values of stem cellulose, its derivative and stem water, Sternberg et al. (2007) observed that $\delta^{18}$OPG was highly correlated with $\delta^{18}$OS, but the correlation between $\delta^{18}$OC and $\delta^{18}$OS was lower because of the isotopic noise in the oxygen attached to the second carbon of the glucose moieties (Fig. 3). Therefore, it is expected that the use of $\delta^{18}$OPG (which does not carry this isotopic noise) will be superior to use of $\delta^{18}$OC in tree-ring studies. Two observations confirm this expectation. First, it can be shown that there is a much better fit ($r = 0.89, P < 0.05$) between $\delta^{18}$OPG as the dependent variable and $\delta^{18}$O and RH as the independent variables compared with using $\delta^{18}$OC as the dependent variable ($r = 0.78, P < 0.05$; Fig. 4). Secondly, by constructing a linear equation using generic values of parameters for the tree-ring model (see Sternberg et al., 2007 for these values), it was shown that the linear factors for the mechanistic equation predicting $\delta^{18}$OPG as a function of $\delta^{18}$O and RH are indistinguishable from those of the best fit linear regression equation (Sternberg et al., 2007). By contrast, the factors of the mechanistic linear equation predicting $\delta^{18}$OC as a function of $\delta^{18}$O and RH are statistically different from those of the best fit equation. Therefore, we cannot reconcile the mechanistic model predicting $\delta^{18}$O values of cellulose...

Fig. 3 Oxygen isotope ratios of (a) stem cellulose ($\delta^{18}$OC), (b) phenylglucosazone ($\delta^{18}$OPG) and (c) oxygen attached to the second carbon of the glucose moieties ($\delta^{18}$OC-2) as a function of the oxygen isotope ratios of stem water ($\delta^{18}$OS). Open symbols represent isotope ratios for samples collected in Arizona where the relative humidity was very low. Regression lines are: $\delta^{18}$OC = (0.52 × $\delta^{18}$OS) + 31.6 with $r = 0.76$ and $P < 0.05$, $\delta^{18}$OPG = (0.73 × $\delta^{18}$OS) + 30.1 with $r = 0.90$ and $P < 0.05$, and (not shown) $\delta^{18}$OC-2 = (−0.30 × $\delta^{18}$OS) + 37.6 with $r = 0.14$ and $P > 0.05$.

Fig. 4 Observed oxygen isotope ratios of cellulose ($\delta^{18}$OC) and phenylglucosazone ($\delta^{18}$OPG) versus that predicted by a best fit multiple linear regression model using $\delta^{18}$O and relative humidity (RH) as independent variables for each of the above chemical components. The multilinear regression prediction equations are: $\delta^{18}$OC = (41.73) − (10.63 × RH) + (0.948 × $\delta^{18}$O) with $r = 0.78$ and $P < 0.05$, $\delta^{18}$OPG = (43.87) − (17.47 × RH) + (1.076 × $\delta^{18}$O) with $r = 0.89$ and $P < 0.05$. 
based on $\delta^{18}O_3$ and RH with a best fit approach. The use of $\delta^{18}O_{P_g}$ values of cellulose, however, successfully reconciles the best fit approach of the first phase with the mechanistic tree-ring model of the second phase.

Future directions

The tree-ring model has been improved by exploring the validity of the common assumptions adopted during the development of the model. Leaf temperature may vary much less than was thought and derivation of cellulose to phenylglucosazone removes biochemically based isotopic noise from the cellulose molecule. There are several other assumptions of the tree-ring model that need to be further explored and may lead to even more improvements. Below, I comment on some assumptions that are particularly critical in further development of the tree-ring model.

Leaf water is under steady state

This assumption has been particularly relevant in partitioning canopy evapotranspiration into transpiration and evaporation using isotope ratios of water vapor over canopies (Lai et al., 2006). It is possible that transpiration is well under way and the leaf has entered steady state by the time carbohydrates are being translocated to tree-ring cellulose synthesis. In a study of oxygen and carbon isotope ratios of the Tasmanian blue gum (Eucalyptus globulus), Cernusak et al. (2005) compared the diurnal variation of the observed $\delta^{18}O_3$ with steady- and nonsteady-state models. The observed $\delta^{18}O_3$ values were in close agreement with both models during the day. However, large differences between observed $\delta^{18}O_3$ values and the steady-state model were seen at night. Therefore, even though there is a discrepancy between steady- and nonsteady-state models in E. globulus, this discrepancy is of no consequence in terms of photosynthate production and the above assumption holds. The validity of the above assumption must also be verified with other species.

The $\delta^{18}O$ value of cellulose from a tree ring is solely determined by the average $\delta^{18}O$ value of that year’s photosynthate

This assumption may not be valid, at least for some species. Empirical correlations between $\delta^{18}O$ and $\delta^{13}C$ values of tree-ring cellulose and environmental factors have shown that the correlation often improves with the inclusion of parameters from the year previous to that represented by the tree-ring cellulose (Reynolds-Henne et al., 2007). $^{13}CO_2$ pulse labeling experiments indicate that the label can persist in cambial starch for up to 3 yr in slow-growing trees such as Larix gmelinii (Kagawa et al., 2006). Kagawa et al. (2006) observed that the carbon isotope ratios in earlywood had the isotopic signal from the summer and autumn photosynthates of the previous year, while latewood seemed to have the climatic signal of the current year's photosynthate. These elegant experiments explain previous observations of the highly significant correlation between the carbon isotope ratio of earlywood and that of the latewood from the previous year (Robertson et al., 1997). However, because there is considerable exchange of oxygen during heterotrophic cellulose synthesis, it is likely that the $^{18}O$ signal of the previous year is dampened off as it is translocated towards current-year earlywood. Pulse labeling experiments similar to those of Kagawa et al. (2006), but using $^{18}O$-enriched water, may be fruitful in determining the influence of the $^{18}O$ signal of previous years in earlywood.

The $\delta^{18}O$ value of leaf water is sensitive to RH

Given the current theory on $\delta^{18}O$ values of leaf water, $\delta^{18}O_L$ may be more sensitive to stomatal response to environmental factors other than RH. Stomatal closure, caused for example by drought, could lead to a greater back flow of the evaporative pool of water in the leaf and cause an overall enrichment of leaf water (Cuntz et al., 2007). In addition, stomatal closure could lead to higher leaf temperatures and increase the vapor pressure gradient across the leaf. One beautiful example where $^{18}O$ in tree-ring cellulose reacts to stomatal closure rather than RH was recently reported by Brooks & Coulombe (in press). Nitrogen fertilization in a stand of Pseudotsuga menziesii caused a flush of leaf growth which increased the foliar to root area ratio and stomatal sensitivity. This higher stomatal sensitivity was reflected in the $\delta^{18}O_C$ of tree rings subsequent to fertilization. Further studies should concentrate on disentangling the actual RH signal from the stomatal response signal.

The $\delta^{18}O$ value of cellulose is solely determined by the water available to the plant

There are no experiments that have been able to demonstrate this. The slope between the $\delta^{18}O$ values of cellulose and those of the water available for photosynthesis in aquarium experiments is always $< 1$ (Cooper & DeNiro, 1989; Yakir & DeNiro, 1990; Sauer et al., 2001), whereas, if the above assumption is valid, one would expect a slope of 1. Several hypotheses have been proposed regarding possible alternate sources of oxygen for cellulose synthesis, such as $O_2$ from photosynthesis or respiration, which was rejected by Cooper & DeNiro (1989), lack of equilibrium between $CO_2$ and water before entering photosynthesis (Cooper & DeNiro, 1989; Yakir, 1992) and the isotopic effect of metabolic water (Sternberg et al., 2007).

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