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A mechanistic model for interpretation of hydrogen and oxygen isotope ratios in tree-ring cellulose

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Abstract—A mechanistic model is presented to quantify both the physical and biochemical fractionation events associated with hydrogen and oxygen isotope ratios in tree-ring cellulose. The model predicts the isotope ratios of tree-rings, incorporating both humidity and source water environmental information. Components of the model include (1) hydrogen and oxygen isotope effects associated with leaf water enrichment; (2) incorporation of leaf water isotope ratio values into photosynthetic carbohydrates along with the biochemical fractionation associated with autotrophic synthesis; (3) transport of exported carbohydrates (such as sucrose) from leaves to developing xylem in shoots and stems where cellulose is formed; (4) a partial exchange of oxygen and hydrogen isotopes in carbohydrates with xylem sap water during conversion into cellulose; and (5) a biochemical fractionation associated with cellulose synthesis. A modified version of the Craig–Gordon model for evaporative enrichment adequately described leaf water δD and δ^{18}O values. The leaf water model was robust over a wide range of leaf waters for both controlled experiments and field studies, far exceeding the range of values to be expected under natural conditions. The isotopic composition of cellulose was modeled using heterotrophic and autotrophic fractionation factors from the literature as well as the experimentally derived proportions of H and O that undergo exchange with xylem water during cellulose synthesis in xylem cells of tree-rings. The fraction of H and O from carbohydrates that exchange with xylem sap water was estimated to be 0.36 and 0.42, respectively. The proportions were based on controlled, long-term greenhouse experiments and field studies where the variations in the δD and δ^{18}O of tree-ring cellulose were measured under different source water isotopic compositions. The model prediction that tree-ring cellulose contains information on environmental water source and atmospheric vapor pressure deficit (related to relative humidity) was tested under both field and greenhouse conditions. This model was compared to existing models to explain cellulose isotope ratios under a wide range of source water and humidity conditions. Predictions from our model were consistent with observations, whereas other models showed large discrepancies as soon as the isotope ratios of source water and atmospheric water deviated from each other. Our model resolves the apparently conflicting and disparate interpretations of several previous cellulose stable isotope ratio studies. *Copyright © 1999 Elsevier Science Ltd*

1. INTRODUCTION

The isotope ratios of meteoric water have been used extensively to reconstruct past climates and in particular, past temperatures from both polar ice cores (Becker et al., 1991; Epstein, 1995) and the annual rings of long-lived trees (Schiegl, 1974; Gray and Thompson, 1976; Epstein and Yapp, 1977; Edwards et al., 1985; Lipp et al., 1991; Feng and Epstein, 1994). Although tree-ring records do not go as far back as ice cores, they contain precisely dated, high-resolution information for up to several thousand years (Fritts, 1976; Fritts and Swetnam, 1989; Switsur and Waterhouse, 1998). Tree-rings may also contain additional intraseasonal information not found in the precipitation records of ice cores related to biological processes that respond to local environmental variation. However, there has been substantial controversy over exactly what biological and environmental information is recorded in the isotopic composition of tree-ring cellulose (e.g., DeNiro and Cooper, 1990; Edwards, 1990). For example, some studies contend that humidity information is recorded in tree-ring cellulose (Yapp and Epstein, 1982; Edwards and Fritz, 1986; Lipp et al., 1993), whereas other studies find no evidence for a humidity signal (DeNiro and Cooper, 1989; White et al., 1994; Terwilliger and DeNiro, 1995). The strong correlation between source water isotope ratio and temperature has lead many investigators to suggest tree-rings as a recorder of environmental temperature (Gray and Thompson, 1976; Epstein and Yapp, 1977; Yapp and Epstein, 1982; Feng and Epstein, 1994). Because stable isotopes are faithful recorders of fractionation events associated with both physical and biochemical processes, the question then becomes, how can interpretation of these apparently disparate results be reconciled?

Most isotopic studies have concluded that tree-rings record environmental information. Generally these studies have used correlations of the measured or assumed plant water source and local weather records with the isotopic composition of cellulose in the corresponding xylem cells in annual growth rings. Typically leaf water and other isotopic parameters (atmosphere and source water) have not been simultaneously measured, because the tree-ring materials available were generally dried tree-ring slabs. Interpretation of such data is potentially biased by the assumptions made and the choice of environmental information

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to be included in the analysis. Climatic reconstruction from tree-rings have relied on correlations between the isotopic composition of tree-ring records and known temperature variation (Schiegl, 1974; Gray and Thompson, 1976; Epstein and Krishnamurthy, 1990), where it is assumed that precipitation is the source water for the tree and the stable isotopes of precipitation vary with condensation temperature (Dansgaard, 1964). A hidden assumption in all of these studies is that the isotope ratios of precipitation reflect average climatic temperatures and not differences in the proportions of moisture derived from winter (cold) versus summer (warm) precipitation events. A number of studies have compared the isotopic composition of tree-ring cellulose and meteoric source water, often yielding regressions with slopes very close to 1.0 (Epstein and Yapp, 1977; Burk and Stuiver, 1981; Yapp and Epstein, 1982; Lawrence and White, 1984; White et al., 1994). However, tree-rings are not likely to be a direct recorder of the isotopic composition of precipitation as there are many steps along the path from meteoric source water to cellulose.

Although there is no fractionation against hydrogen or oxygen isotopes during water uptake by the roots (White et al., 1985; Ehleringer and Dawson, 1992; Dawson, 1993, although mangrove trees growing in salt water were an exception, Lin and Sternberg, 1993), water in the leaves is isotopically enriched due to evaporation (Craig and Gordon, 1965; Flanagan et al., 1991b; Yakir, 1992). We know that isotopes from the enriched leaf water pool are then incorporated into organic compounds through biochemical processes that also involve fractionation against heavy isotopes (Sternberg, 1989; Luo and Sternberg, 1992; Yakir, 1992; Farquhar et al., 1998). The questions then become "when these carbohydrates are then transported from the leaf to the site of cellulose synthesis in the stem, are there further fractionation events?" and "is isotopic exchange with xylem water possible?" The tortuous path from precipitation to tree-ring cellulose suggests that correlation studies may not be sufficient to answer some of these nagging questions regarding the information contained within the tree-ring and that experimental approaches are needed.

Unfortunately, no single experimental study other than Roden and Ehleringer (1999a) (to be discussed later) has addressed both exchange processes associated with hydrogen and oxygen isotope ratios in tree-ring cellulose. Previous short-term experimental studies that addressed the mechanisms by which hydrogen or oxygen isotopes of water were incorporated into cellulose have limited applications to tree-rings for a variety of reasons. DeNiro and Cooper (1989) using potato and Terwilliger and DeNiro (1995) using avocado concluded that at the time of cellulose synthesis there is a reequilibration with the medium (xylem) water, eliminating the effects of previous fractionation events and making the isotopic composition of cellulose directly related to that of source water. However, cellulose synthesis in both developing potato shoots and avocado seedling shoots may differ from that which takes place in the cambium of a tree due to differences in the substrate (storage organic molecules) leading up to the sugar, which is then transported to the developing xylem cell. Both potato and avocado systems use substantial amounts of stored carbon sources, which may have different δD and $\delta^{18}O$ values than currently produced substrates being transported from the leaf. In addition, in short-term experiments (weeks), there are also concerns regarding experiment length, because of the history of possibly different storage sources that would contribute to the small amounts of biomass formed in the experiments or because of the possibility that in developing, nonsuberized stem and tuber tissues, evaporative enrichment could occur in the xylem water (Dawson and Ehleringer, 1993). White et al. (1994) modeled tree-ring hydrogen isotope ratios based on several years of field observations of tree-rings and where essential factors influencing the leaf water isotope ratio had been monitored. Given the range of source water and humidity isotope values in that study, White et al. (1994) were not able to distinguish between a model in which humidity effects were incorporated into cellulose versus a model in which there was complete reequilibration with source water before cellulose formation. Perhaps the most mechanistic and appropriate study to date was conducted by Yakir and DeNiro (1990), who studied leaf cellulose in *Lemna gibba* L., a water fern, under a range of source water values. Unfortunately, stem cellulose is not formed in *Lemna* and therefore, a direct comparison in which sugars are exported from the leaf was not possible.

A mechanistic, plant physiologic understanding of how information from the hydrogen and oxygen isotopes of water are incorporated into tree-ring cellulose is needed to resolve this issue. If there is incomplete exchange between substrate molecules and medium water at the time of cellulose synthesis in xylem cells of tree-rings, then the model must explain the commonly observed 1:1 relationship between the isotopic composition of source water and tree-ring cellulose. However, if there is complete exchange at the time of cellulose synthesis in xylem cells of tree-rings, then the model must explain the commonly observed correlations between xylem cellulose isotopic composition and humidity. Furthermore, the model must account for potential variations in the isotopic composition of cellulose formed in leaves, shoots, and roots. Last, the model must account for isotope effects across a broad range of climatic conditions, including growth regimes where the isotopic composition of water in the soil and in the atmosphere are not in equilibrium with each other.

Here we present the model and experimental results that allow a direct comparison between predicted and measured values of both leaf water and cellulose for both oxygen and hydrogen isotope ratios in tree-rings.

2. THE MODEL

Our model is based on the premise that (1) the hydrogen and oxygen atoms incorporated into organic molecules during photosynthesis take on an isotopic signature related to that of leaf water and that (2) a fraction of those atoms later exchange with medium water during cellulose synthesis, regardless of whether that cellulose synthesis occurs in leaves, stems, or roots. Although parts of this model have been described before by other researchers, a complete picture, aspects of the potential biochemical exchange, and a mechanistic explanation have been lacking. In addition, no previous modeling effort has linked both hydrogen and oxygen isotope ratios into a single model.

Our modelling effort has two major components: (1) a leaf water model that uses environmental parameters to predict the extent of evaporative enrichment for a given input of source water and atmospheric vapor and (2) a biochemical model that

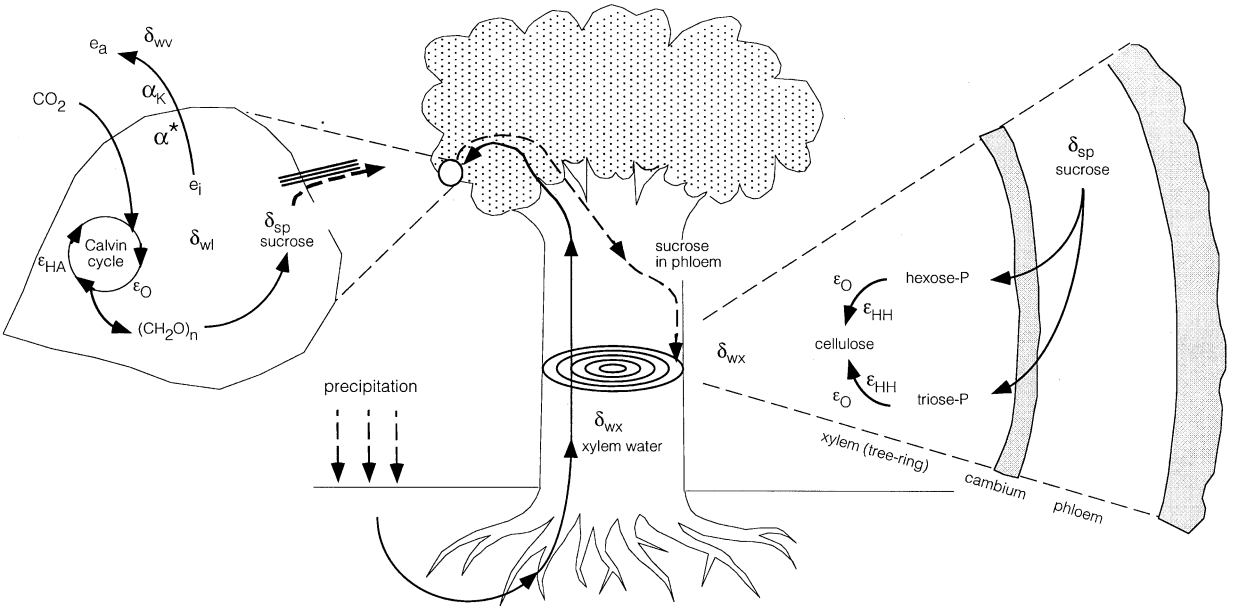

Fig. 1. A diagram of the isotopic fractionation events occurring between precipitation input and tree-ring cellulose.

predicts cellulose isotopic composition based on the extent of isotopic exchange between organic components in the steps leading to cellulose synthesis as well as incorporating known autotrophic and heterotrophic fractionation factors.

The hydrogen atoms of cellulose are typically thought of as originating from the water taken up by tree roots (Fig. 1). Although both water and carbon dioxide provide oxygen atoms in plant carbohydrate metabolism, the oxygen originating in CO_2 exchanges completely with water before carbohydrate synthesis (DeNiro and Epstein, 1979). Thus, the δD and δ^{18}O values carried through the process of cellulose production might be thought of as representing, to a first approximation, the integrated water uptake patterns of the root system. However, it is not so straightforward, as there are numerous points along the pathway between water uptake through the root and cellulose formation into a tree-ring where the hydrogen and oxygen isotopic composition of either water or organic molecules may be altered.

We expect that water within leaves is isotopically enriched in hydrogen and oxygen, primarily as a consequence of evaporation. As shown in Figure 1, this enriched isotopic signal is incorporated into initial photosynthetic products. Further biochemical fractionation involving hydrogen and oxygen in leaves also occurs during sugar synthesis. At this point, the photosynthetic sugar product can either be exported to other parts of a plant, normally as sucrose in most plants, or stored temporarily within the chloroplast as starch. We assume that there is no net fractionation associated with starch formation and subsequent degradation, as all of the starch/sugar product substrates are consumed. When the sucrose is broken down within a developing cell to form cellulose, isotopic exchange can occur. We predict that the extent of that isotopic exchange depends on the fraction of the sugars that breaks down into triose phosphates before becoming synthesized into cellulose, equilibrium isotope exchange between these organic molecules and the surrounding medium water, and on biochemical fractionation events associated with cellulose synthesis (Fig. 1).

Throughout this article we use conventional "delta" notation, where the isotopic composition of a material relative to that of a standard on a per mil deviation basis is given by,

$$\delta = \left(\frac{R_{sample}}{R_{standard}} - 1 \right) \cdot 1000, \quad (1)$$

where δ is the isotope ratio (δD for hydrogen and δ^{18}O for oxygen) and R is the molar ratio of heavy to light isotope forms. The standard for both hydrogen and oxygen is standard mean ocean water (SMOW; Appendix 1).

2.1. Leaf Water Enrichment

A model for the evaporative enrichment for both δD and δ^{18}O of open water surfaces was developed by Craig and Gordon (1965). Flanagan et al. (1991b, see also Farquhar et al., 1989) expanded the Craig–Gordon model to include leaf boundary layer considerations and diffusion through stomata, making it more appropriate for modeling leaf water:

$$R_{wl} = \alpha^* \left[\alpha_k R_{wx} \left(\frac{e_i - e_s}{e_i} \right) + \alpha_{kb} R_{wx} \left(\frac{e_s - e_a}{e_i} \right) + R_a \left(\frac{e_a}{e_i} \right) \right], \quad (2)$$

where subscripts wl, a, i, s, and wx refer to leaf water, bulk air, intercellular air spaces, leaf surface, and xylem water (same as source water), respectively. The fractionation factors differ depending on whether hydrogen or oxygen isotopes are being modeled, but the same general model applies for both species. α^* is the liquid–vapor equilibrium fractionation factor and varies with temperature according to the equations of Majoube (1971) for both H/D and $^{16}O/^{18}O$, α_k is the kinetic fractionation associated with diffusion in air (H/D = 1.025 and $^{16}O/^{18}O$ =

1.0285), and α_{kb} is the kinetic fractionation associated with diffusion through the boundary layer and is calculated by increasing α_k to the 2/3 power (H/D = 1.017 and $^{16}O/^{18}O$ = 1.0189).

The environmental factors influencing leaf water isotope composition are the δ values of the water taken up by the roots, as well as the δD and $\delta^{18}O$ of atmospheric vapor and the vapor pressures of the intercellular air spaces of the leaf (e_i), the leaf surface (e_s), and the bulk air (e_a). In only one aspect of the leaf water calculations do leaf physiologic characteristics come into consideration. This is in the calculation of the vapor pressure at the leaf surface, e_s, which is estimated using stomatal conductance and transpiration rate as in Ball (1987),

$$w_s = \frac{g_{sw} \cdot w_i - E(1 - w_i/2)}{g_{sw} - E/2}, \quad (3)$$

where w_s is the mole fraction of water vapor at the leaf surface, w_i is the mole fraction of intercellular water vapor, g_{sw} is stomatal conductance to water vapor, E is transpiration rate. w_s is multiplied by atmospheric pressure to obtain e_s. This equation was used as an approximation to the mathematically correct ternary corrections introduced by Caemmerer and Farquhar (1981) due to the lack of field data on intercellular CO_2 concentrations. Sensitivity analysis shows that Eqn. 2 is not sensitive to changes in e_s. Only very large (order of magnitude) differences in the estimation of stomatal conductance or transpiration rate will effect the δD and $\delta^{18}O$ estimations from Eqn. 2, and then by only a few ‰.

Eqn. 2 predicts the δD and $\delta^{18}O$ values of water at the site of evaporation, which we assume to be equivalent to the isotopic composition of water at the site of carbohydrate metabolism (the chloroplast). The proximity of the chloroplast to the air–water interface and the results of Flanagan et al. (1994) lend support to this assumption (although Yakir et al., 1993, have shown contrasting results). The Craig–Gordon model contains a number of assumptions that may not be strictly valid for leaves such as isotopic steady state, constant water volume, and isotopic homogeneity (Flanagan, 1993; Yakir, 1998). Many studies (Allison et al., 1985; Leaney et al., 1985; Flanagan et al., 1991a; Flanagan and Ehleringer, 1991; Wang and Yakir, 1995) have found that the Craig–Gordon model predicted greater isotopic enrichment than was observed in bulk leaf water. Thus, a correction is needed when testing the model against bulk leaf water measurements to account for the many possible factors that could influence bulk leaf water,

$$\delta D_{bulk} = \delta D_{wl} \cdot f_l + \delta D_{wx} \cdot (1 - f_l), \quad (4)$$

$$\delta^{18}O_{bulk} = \delta^{18}O_{wl} \cdot f_l + \delta^{18}O_{wx} \cdot (1 - f_l), \quad (5)$$

where f_l is the proportion of the bulk leaf water subjected to evaporative enrichment and the subscript bulk, wl and wx refers to bulk leaf water, leaf water at the site of evaporation (Eqn. 2) and xylem water, respectively. Spatial heterogeneity in δD and $\delta^{18}O$ values of water within a leaf have been observed in leaves (Yakir et al., 1989; Luo and Sternberg, 1992; Wang and Yakir, 1995). Although this heterogeneity may be accounted for by anatomic studies that calculate the proportion of the overall volume occupied by large veins (Flanagan et al., 1991b), these measurements are laborious and may not account for all the heterogeneity in the leaf. Farquhar and Lloyd (1993) have developed an equation to account for leaf heterogeneity that describes the convection of xylem water that is opposed by back diffusion of the isotopically enriched water from the site of evaporation (the Péclet effect). The Péclet correction requires knowledge of the effective mixing length, which, in practice, is estimated empirically from the discrepancies between the Craig–Gordon model and measured bulk leaf water measurements. In addition, some researchers have found that including leaf morphologic and physiologic characteristics can enhance predictions of the isotopic composition of leaf water (Buhay et al., 1996; Wang et al., 1998). However, some heterogeneity may still be unaccounted for due to patchy stomatal conductances (Mott, 1995), which is difficult to model. Because of the many and potentially interacting effects described previously, we choose to bundle them all together into empirical equations (Flanagan, 1993) that correct bulk leaf water measurements. Throughout this article we use the term leaf water to indicate the site of evaporation (δ_{wl}), which is what is needed for cellulose modelling, and the term bulk leaf water for measurements derived from whole leaf extractions of water.

Two points need to be considered with respect to possible biochemical fractionation in the steps leading to cellulose synthesis. First, what are the fractionation factors? Second, what fraction of the atoms in an organic molecule is subject to exchange with the local medium water during synthesis?

2.2. Biochemical Fractionation Factors

Photosynthesis strongly influences the δD values of organic matter. Within the chloroplast, photosynthetic electron transport discriminates against D and forms a pool of available reductant that is depleted in the heavy isotope (negative δD values) (Estep and Hoering, 1981), due primarily to a reduced dissociation of DHO as compared to H_2O (Luo et al., 1991). Sugars produced during photosynthesis within the chloroplast are lighter than the surrounding leaf water (which is already enriched relative to soil water as presented in Eqn. 2). Yakir and DeNiro (1990) calculated that the hydrogen isotope fractionation factor associated with autotrophic carbohydrate metabolism (ϵ_{HA}) was −171‰. Estep and Hoering (1981) estimated a slightly different value for autotrophic fractionation (−100 to −120‰) for microalgae. In contrast to the negative biological fractionation associated with autotrophic metabolism, the hydrogen isotope fractionation associated with heterotrophic carbohydrate metabolism, ϵ_{HH}, is +158‰ for *Lemna* (Yakir and DeNiro, 1990). Luo and Sternberg (1992) reported nearly identical ϵ_{HH} values (+144 to +166‰) for *Hordeum*, *Triticum*, and *Ricinus*.

The oxygen isotope ratio values within biochemical components of a plant are far less variable than the δD values (Sternberg, 1989; Yakir, 1992). Although oxygen could potentially come from sources other than water, DeNiro and Epstein (1979) showed that oxygen in CO_2 undergoes complete exchange with water before fixation in organic compounds. The $\delta^{18}O$ values of leaf cellulose are +27‰ elevated above leaf water (Sternberg, 1989; Yakir and DeNiro, 1990). The mechanism for the 27‰ enrichment is the carbonyl–water interaction during biosynthesis (Sternberg and DeNiro, 1983). Because the fractionation factor is the same for autotrophic and

heterotrophic fractionation of oxygen, there is no need to distinguish between the two ($\epsilon_O = +27‰$).

Yakir and DeNiro (1990) showed that postphotosynthetic metabolism affected the δD value of cellulose and observed that about 50% of the carbon-bound hydrogen in leaf cellulose was exchanged with hydrogen in water during cellulose synthesis. Although carbon-bound hydrogens in organic matter do not exchange with their environment (Epstein et al., 1976), there are numerous opportunities to do so during many biosynthetic reactions, such as those involving NADP, isomerases, reductases, kinases and carboxylases (Luo and Sternberg, 1991, 1992; Yakir, 1992).

2.3. Isotopic Exchange During Cellulose Synthesis

After the original incorporation of a leaf water and autotrophic fractionation into photosynthetic sugars, a second set of fractionation can occur if there is a reequilibrium exchange between the carbohydrate and local medium water (leaf water in the case of leaf cellulose, or xylem water in the case of tree-ring xylem cellulose synthesis). Sternberg et al. (1986), Yakir and DeNiro (1990), and Luo and Sternberg (1992) proposed that there was only a partial isotopic exchange during cellulose synthesis. Their general models for the δD and $\delta^{18}O$ values of heterotrophically produced leaf cellulose versus the respective δD and $\delta^{18}O$ values of source water were of the form

$$\delta_c = f \cdot (\delta_w + \varepsilon) + (1 - f) \cdot \delta_{\text{non-exchangeable}}, \quad (6)$$

where the subscripts c, w, and nonexchangeable indicate the δD and $\delta^{18}O$ values of synthesized cellulose, medium water, and nonexchangeable stable isotopes of the substrate, respectively, ϵ is the isotope fractionation factor for the enzyme-mediated exchange or addition of either hydrogen or oxygen, and f is the proportion of the carbon-bound hydrogen or oxygen that undergoes exchange with medium water. Yakir and DeNiro (1990) investigated the water fern *Lemna* growing under heterotrophic conditions in the dark (sucrose fed). They reported that $f \approx 35\%$ for both oxygen and nonexchangeable hydrogen in leaf cellulose.

The isotopic composition of cellulose is predicted to be a function of both the isotope ratio of the substrate sucrose and of the medium water within the cell, which could be a leaf cell, a root cell, or a stem xylem cell as part of a tree-ring. Tree-ring cellulose isotope ratio (δD_{cx} and $\delta^{18}O_{cx}$) is then calculated as;

$$\delta D_{cx} = f_H \cdot (\delta D_{wx} + \varepsilon_{HH}) + (1 - f_H) \cdot (\delta D_{wl} + \varepsilon_{HA}), \quad (7)$$

$$\delta^{18}O_{cx} = f_O \cdot (\delta^{18}O_{wx} + \varepsilon_O) + (1 - f_O) \cdot (\delta^{18}O_{wl} + \varepsilon_O), \quad (8)$$

where f_H and f_O refer to the fraction of carbon-bound hydrogen and oxygen, respectively, that undergoes exchange with medium water and the subscript wl indicates the leaf water value at the site of sucrose synthesis and is obtained from either the leaf water model (Eqn. 2) or from bulk leaf water measurements that have been corrected for the proportion of tissues that were not exposed to evaporative enrichment (Eqns. 4 and 5). This model predicts that given the opposite signs of ϵ_{HA} and ϵ_{HH} xylem cellulose isotope ratio values (δD_{cx}) should approach xylem water values (δD_{wx}). This similarity alone may explain the commonly observed correlations of meteoric water source and tree-ring cellulose seen by many studies. This proposed model also predicts that δD_{cx} should vary with humidity as has been seen from late-wood studies cited previously. If the isotopic values of atmospheric water and source water are in near equilibrium with each other, it is very difficult to distinguish between a model with complete isotopic exchange during cellulose synthesis (in which case the humidity signal is lost) from our model in which there is only partial isotopic exchange during cellulose synthesis (in which case the humidity signal is at least partially retained).

In a similar fashion to cellulose, the sucrose exported from the leaf through the phloem (δD_{sp}) may also exchange with leaf water;

$$\delta D_{sp} = f_H \cdot (\delta D_{wl} + \varepsilon_{HH}) + (1 - f_H) \cdot (\delta D_{wl} + \varepsilon_{HA}), \quad (9)$$

$$\delta^{18}O_{sp} = f_O \cdot (\delta^{18}O_{wl} + \varepsilon_O) + (1 - f_O) \cdot (\delta^{18}O_{wl} + \varepsilon_O). \quad (10)$$

3. METHODS AND MATERIALS

All results presented were compiled from three separate long-term field or greenhouse experiments (Roden and Ehleringer, 1999a, 1999b, 1999c). The data are brought together here to provide an evaluation of the model based on different experimental designs (i.e., controlled environments versus field-based experiments). Thus, full details of the experimental design and methodology are not presented here.

Three tree species were used for the long-term observations. All were either saplings (2 m tall) obtained from nurseries (controlled experiments) or young (<10 cm diameter) trees growing immediately adjacent to a river (field experiments). The species in the studies were alder (*Alnus incana*, or *A. rubra*), birch (*Betula occidentalis*), and cottonwood (*Populus fremontii*, or *P. angustifolia*). In the controlled experiments, plants were grown in hydroponic solutions (Roden and Ehleringer, 1999a, 1999b) with different but constant source water isotopic compositions. The field sites (coastal Oregon, central Arizona, and northern Utah riparian zones) were chosen for their variation in river water isotopic composition and variation in relative humidity (Roden and Ehleringer, 1999c). The gas exchange measurements are described in detail in Roden and Ehleringer (1999b).

Source water used by the tree was obtained from either sampling the hydroponic tank water (greenhouse studies) or cutting suberized stems (field studies) and extracting water cryogenically as in Ehleringer and Osmond (1989). Leaf water was also extracted cryogenically from upper canopy leaves under both field and greenhouse observations. Atmospheric water vapor was sampled by drawing air through an ethanol/dry ice trap. The current year's tree-ring was cut from either harvested stems or from increment cores. Temperature and humidity measurements were made at the time of leaf water sampling.

Field studies were conducted in Arizona, Oregon, and Utah, where alders, birch, and cottonwood occur naturally along stream banks. The field studies relied on less intensive measurements than the greenhouse conditions Roden and Ehleringer (1999c). Air temperature, humidity, atmospheric water vapor, source water, and leaf water were collected monthly throughout the growing season. Transpiration rates, stomatal and leaf boundary layer conductance were not measured in the field and greenhouse estimates were used in the modeling exercise. However, a sensitivity analysis showed that even an order of magnitude change in transpiration rate, stomatal conductance, or boundary layer conductance would affect estimates of leaf water δD values by only 2 to 3‰. Thus, we believed that using greenhouse gas exchange values for the field simulations was acceptable.

Samples were prepared for mass spectrometric analysis by one of several methods. The δD values of water samples were obtained by reduction with zinc (Coleman et al., 1982), but modified so that the reaction occurred at 420°C. The $\delta^{18}O$ values of water were obtained by equilibration with CO_2 (Socki et al. 1992). For the on-line gas exchange measurements, we further reduced the volumes described in Socki et al. (1992) to accommodate the small water volume (typically

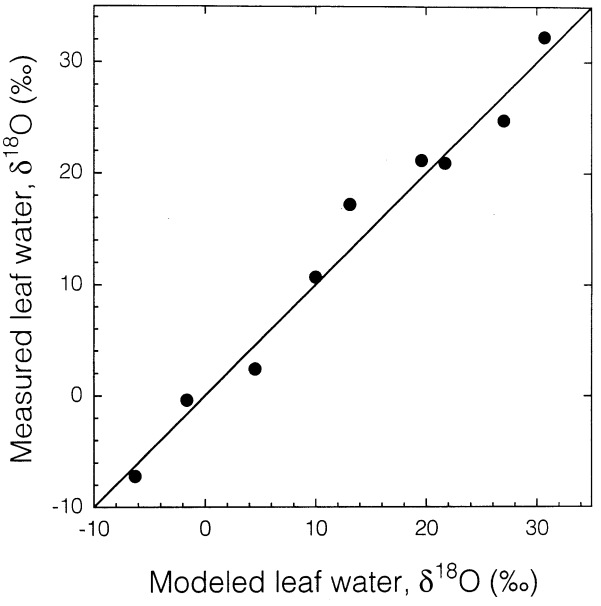

Fig. 2. The relationship between the $\delta^{18}O$ of modeled and measured leaf water. Variations in leaf water were generated in a gas exchange cuvette through altering input vapor $\delta^{18}O$, source water $\delta^{18}O$, as well as vapor pressure deficits and flow rates. The line represents a 1:1 relationship. From Roden and Ehleringer (1999b).

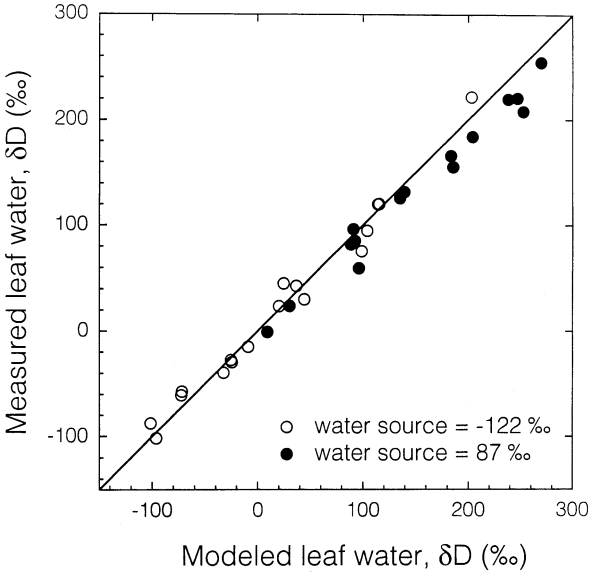

Fig. 3. The relationship between the δD of modeled and measured leaf water. Variations in leaf water were generated in a gas exchange cuvette through altering input vapor δD, source water δD, as well as vapor pressure deficits and flow rates. The line represents a 1:1 relationship. From Roden and Ehleringer (1999b).

40 to 60 μL). The $\delta^{18}O$ of cellulose from tree-rings was obtained by on-line pyrolysis (Saurer et al., 1998) of α-cellulose derived using the methods described by Leavitt and Danzer (1992). The δD values of cellulose from tree-rings was obtained by first combusting nitrated α-cellulose in the presence of cupric oxide to obtain water. Hydrogen gas was then produced from the water of combustion using the Coleman et al. (1982) methods referred to earlier. All samples were analyzed on a Finnigan MAT delta S isotope ratio mass spectrometer. Typical overall precision was ±1‰ for δD values and ±0.2‰ for $\delta^{18}O$ values.

Data are presented as plots of the modeled versus measured isotopic compositions as compared to a 1:1 line. Data relating water sources, environmental, and other measurements can be found in Roden and Ehleringer (1999a, 1999b, 1999c). The on-line gas exchange system continuously recorded all the parameters needed for the leaf water model, including temperature, humidity, stomatal conductance, and transpiration rate. The gas exchange system allowed testing of the model over a wide range of leaf waters (≈400‰ for δD and 40‰ for $\delta^{18}O$) and had the benefit of allowing the leaf to reach steady-state (an important assumption in the model) after a minimum of 5 h under constant environmental conditions.

4. RESULTS AND DISCUSSION

4.1. Evaluation of Leaf Water Component of Model

A modified version of the Craig–Gordon model for evaporative enrichment that includes boundary layer considerations (Flanagan et al., 1991b) clearly predicted measured leaf water isotope ratio values across a wide range of controlled environmental conditions for all tree species (Figs. 2 and 3). Irrespective of water source value or humidity characteristics, there was a strong fit of the data to the modified Craig–Gordon model over a 400‰ variation in δD values and a nearly 40‰ variation in $\delta^{18}O$ values. Other studies (Allison et al., 1985; Leaney et al., 1985; Flanagan et al., 1991a; Flanagan and Ehleringer, 1991; Wang and Yakir, 1995) have found that the Craig and Gordon model (1965) predicted somewhat greater isotopic enrichment than was actually observed in leaves. These small discrepancies in earlier studies may have been due in part to insufficiently accounting for the contribution of unfractionated water in leaf tissues (recall Eqns. 4 and 5) or an atmosphere that was sufficiently dynamic that there was not isotopic equilibrium between the leaf and the atmosphere. In our measurements, the portion of unfractionated water (f_1) was 10%, which is slightly lower than the 13 to 33% values reported in other studies (Allison et al., 1985; Leaney et al., 1985; Flanagan et al., 1991b). One potential difference is that in our study, all measurements were collected under equilibrium conditions. Thus, there was no correlation between the deviations of predicted and observed leaf water isotope values and flux rates (transpiration), although transpiration rates were much lower in our tree species than in the species reported by Flanagan et al. (1991b). The f_1 parameter was empirically determined and is an attempt to incorporate all the factors that influence bulk leaf water isotopic heterogeneity (compartmentation, Péclet effect, patchy stomata etc.) for the species tested. The f_1 value of 10% gave excellent agreement for both species over an extended range of leaf water isotopic composition. Thus, it may be possible to determine the f_1 value using detailed microenvironmental and gas exchange measurements as well as the isotopic composition of bulk leaf water for species with similar leaf morphologies, and use that value as a reasonable correction over a wide range of bulk leaf water observations.

There may be some question as to whether or not the modified Craig–Gordon model completely explained the leaf water observations at the most positive δD values (Fig. 3), because there is a potential slight deviation from the 1:1 line. Experimentally, the highly positive values of leaf water δD were generated as a consequence of both artificially enriched water

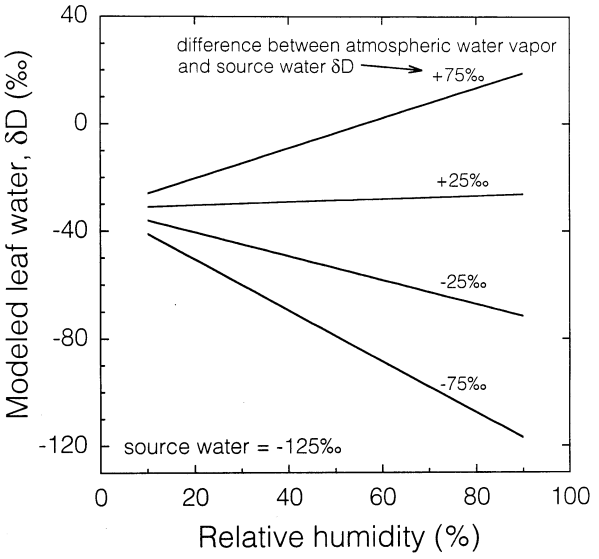

Fig. 4. Sensitivity analysis of the leaf water model to variations in atmospheric vapor δD and relative humidity.

sources and cuvette water vapor inputs. Some small deviations between the modeled and measured leaf water isotope ratios appear at the highly enriched end of the data set and may be due to the sensitivity of the leaf water model to both absolute humidity and atmospheric water vapor δD and $\delta^{18}O$ values. Figure 4 presents results of the model calculations, showing that the leaf water model is very sensitive to humidity, which will become obvious when the differences between water source and atmospheric vapor δD are large. The slope of the line is −0.44 when the difference between source and atmosphere is −25‰ (Fig. 4), implying that an error in humidity measurement of 10% will cause an error in modeled leaf water δD of 4.4‰. However, the error would be 9.5‰ and 5.6‰ for leaves exposed to a difference of −75‰ and +75‰, respectively. Some of the differences between modeled and measured leaf water for highly enriched leaves in Figure 3 may be related to small measurement errors in humidity, which are then compounded by the large differences between source water and atmospheric vapor δD values (some differences were as high as 300‰).

Figure 4 also reveals that the leaf water model is sensitive to measurements of atmospheric vapor δD, and the sensitivity is a function of humidity. At 90% relative humidity, a 10‰ error in the measurement of atmospheric water vapor δD will cause an error in modeled leaf water δD of 8.6‰ but only an error of 2.9‰ at a humidity of 30%. This analysis confirms the conclusions of White et al. (1994) regarding the role of variations in atmospheric δD for the isotopic composition of cellulose and that previous studies using rough estimates of humidity or atmospheric vapor δD may be in error. Care must be taken to ensure that both humidity and atmospheric vapor δD and $\delta^{18}O$ values are measured as accurately as possible and that seemingly small variations in these parameters can have profound leaf water effects. The monitoring of atmospheric water vapor isotope ratios is uncommon relative to the more frequent measurements of the isotopic composition of ground and surface waters. If the interpretation of leaf waters (and ultimately of cellulose values) are to become more common in environmental studies, then it is essential to know more about the short-term variations and the long-term relationships between isotopic composition of atmospheric water vapor and soil water in terrestrial ecosystems.

4.2. Greenhouse and Field Evaluations of Biochemical Fractionation and Reequilibration Components of the Model

The model predicts that sugars formed as a result of photosynthesis in leaves will be exported to the developing tree-ring with an isotope ratio that incorporates the enriched leaf water isotope ratio values, in addition to any hydrogen and oxygen biochemical fractionation factors that occur during synthesis. We did not measure the biochemical fractionation factors directly in this study, but used values from the literature. Autotrophic processes have been recognized as exhibiting high, but consistent fractionation factors. In our model, we used a hydrogen isotope biological fractionation factor for autotrophic carbohydrate metabolism of $\epsilon_{HA} = -171‰$, which is from Yakir and DeNiro (1990). Although this value gave reasonable predictions more work is needed to determine potential variability in ϵ_{HA} for different species. In our model, we used an oxygen isotope biological fractionation factor for autotrophic carbohydrate metabolism of $\epsilon_O = +27‰$. The value of this fractionation factor is derived from Sternberg and DeNiro (1983) and has been confirmed by other studies as well. Thus, the nonexchangeable H and O atoms in sucrose (δ_{sp}) leaving the leaf and being transported in the phloem to the developing tree-ring xylem cells are predicted to be depleted by −171‰ and enriched by 27‰ as compared to leaf water (δ_{wl}) for hydrogen and oxygen isotope ratios, respectively.

A second set of fractionation events is predicted to occur when the sucrose is eventually converted to cellulose in the developing tree-ring xylem cells. Irrespective of the isotopic exchange between the organic molecules and the xylem water, there will be a second set of biochemical fractionation factors associated with the heterotrophic synthesis reactions. Yakir and DeNiro (1990) reported that the hydrogen isotope heterotrophic fractionation factor was $\epsilon_{HH} = +158‰$, similar to the mean multispecies value of +155‰ derived from Luo and Sternberg (1992). The near-equivalent magnitudes and opposite signs of ϵ_{HA} and ϵ_{HH} indicate that the hydrogen isotope ratio values of tree-ring cellulose (δD_{cx}) might be expected to approach that of xylem water (δD_{wx}) as has been commonly observed by many previous studies (Burk and Stuiver, 1981; Epstein and Yapp, 1977; Yapp and Epstein, 1982; Lawrence and White, 1984; White et al., 1994).

If the isotopic composition of stem cellulose is primarily affected by the isotopic composition of water at the site of cellulose synthesis and not by fractionation processes in the leaf as has been claimed by DeNiro and Cooper (1989) and later by Terwilliger and DeNiro (1995), then the isotopic composition of tree-ring cellulose should be similar to that of source water plus biochemical fractionation factors. That is to say, both f_H and f_O should be equal to 1.0 if these previous models were correct. However, our long-term greenhouse observations are not consistent with f_H and f_O equal to 1.0 (Figs.

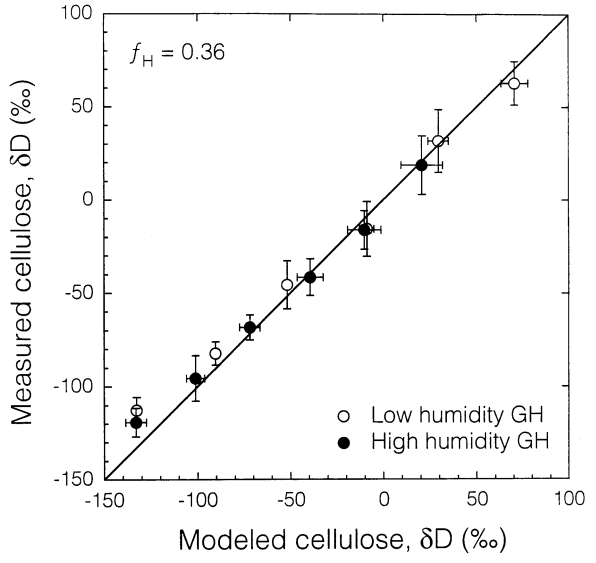

Fig. 5. The relationship between the δD of modeled and measured tree-ring cellulose. Variations in tree-ring cellulose were generated by altering source water δD in a hydroponic system in a controlled greenhouse environment at either high or low relative humidity. Values are means and standard deviations. The line represents a 1:1 relationship. From Roden and Ehleringer (1999a).

5 and 6). Instead we calculated best-fit values of f_H and f_O as 0.36 and 0.42, respectively. These long-term tree-ring synthesis observations are more consistent with the results of short-term studies of germinating seeds by Luo and Sternberg (1992) where cellulose synthesis originated from different organic storage compounds. They observed that (1) f_H and f_O were of roughly similar magnitude and (2) that both f_H and f_O exchange coefficients were much less than 1. When cellulose was derived from starch, Luo and Sternberg (1992) observed that $f_H \approx f_O \approx 0.34$. When cellulose was derived from lipids, $f_H \approx f_O \approx 0.67$ (Luo and Sternberg, 1992). Cellulose synthesis from starch as a starting point is much more biochemically similar to synthesis from sucrose as a starting point. Thus, it is reassuring that the exchange coefficients (f_H and f_O) observed by Luo and Sternberg agree well with our long-term observations. Both results support the prediction that there is an incomplete isotopic exchange between organic molecules and the medium water during cellulose synthesis.

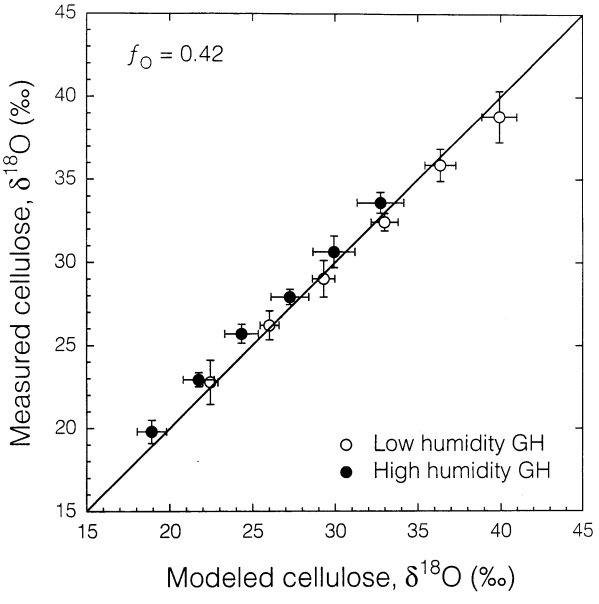

Fig. 6. The relationship between the $\delta^{18}O$ of modeled and measured tree-ring cellulose. Variations in tree-ring cellulose were generated by altering source water $\delta^{18}O$ in a hydroponic system in a controlled greenhouse environment at either high or low relative humidity. Values are means and standard deviations. The line represents a 1:1 relationship. From Roden and Ehleringer (1999a).

For plants grown in the greenhouse hydroponics study (Figs. 5 and 6), the δD and $\delta^{18}O$ of the most enriched cellulose at both low and high humidities were grown with identical water sources (+180‰, δD and +10‰, $\delta^{18}O$). Yet plants in these experiments did not exhibit identical δD and $\delta^{18}O$ values for cellulose. That is, under the most enriched water sources, the observed hydrogen isotope ratios for low-humidity and high-humidity treatments were +63‰ and +19‰ (Fig. 5), respectively. None of these organic isotope ratio values approached the source water values of +175‰ (Fig. 5) for the most enriched experimental treatments. If reequilibration of the isotopes in the organic molecule with xylem water at the time of cellulose synthesis were to have occurred, then clearly the cellulose in the tree-rings would be much different than observed. In addition, the differences between high (≈70%) and low (≈40%) humidities demonstrated that a humidity signal was retained in the tree-ring cellulose, a conclusion that differs from the one of DeNiro and Cooper (1989) and Terwilliger and DeNiro (1995). White et al. (1994) pointed out that their field data did not allow a satisfactory evaluation of whether or not a humidity signal was retained in tree-ring cellulose. The experimental design of our greenhouse hydroponics study (Roden and Ehleringer, 1999a) provides the best opportunity to determine whether or not a humidity signal is retained in cellulose, because (1) plants were grown under two contrasting humidity conditions for an entire growing season and (2) plants grown under different water sources were exposed to a constant atmosphere where the isotopic composition of water vapor was in equilibrium with only one of the six possible water sources. In contrast to previous shorter term studies, these growth regime differences were maintained for an entire growing season, long enough to produce a tree-ring. In those experimental treatments, when atmospheric vapor δD values were similar to that of the source water δD (as it was in the most depleted treatments in Fig. 5, source water = −120‰, water vapor = −150‰), then the cellulose δD value (−115 to −120‰) appear to be similar to source water values, indicating a 1:1 relationship between source water and tree-ring cellulose similar to what has been observed in other studies (Yapp and Epstein, 1982). Differences between source water and cellulose δD values would only become evident when large differences existed in either relative humidity or in the isotopic composition of atmospheric vapor and source water, both of which are essential to create substantial variations in leaf water isotope values.

The model was then used to evaluate leaf water and tree-ring isotope ratios of trees grown under field situations. Despite the

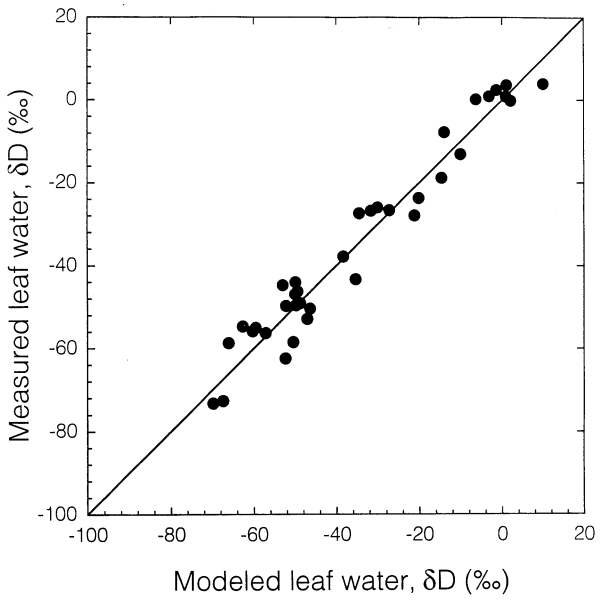

Fig. 7. The relationship between the δD of modeled and measured leaf water for field grown riparian zone trees. Variations in leaf water were due to different stream water δD values and temperature and humidity differences between sites. The line represents a 1:1 relationship. From Roden and Ehleringer (1999c).

absence of precise gas exchange data and discontinuity of environmental data, the predicted δD and $\delta^{18}O$ values for leaf waters agreed well with observed values (Figs. 7 and 8). The increased scatter in the data as compared to the laboratory experiments is likely a result of estimations rather than direct

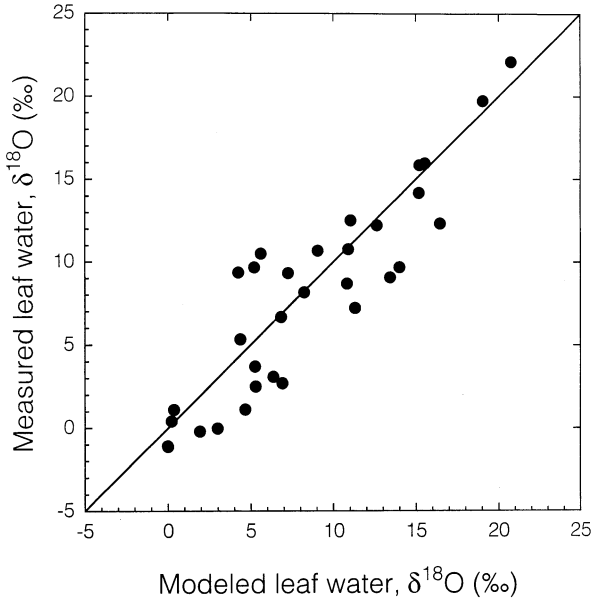

Fig. 8. The relationship between the $\delta^{18}O$ of modeled and measured leaf water for field grown riparian zone trees. Variations in leaf water were due to different stream water $\delta^{18}O$ values and temperature and humidity differences between sites. The line represents a 1:1 relationship. From Roden and Ehleringer (1999c).

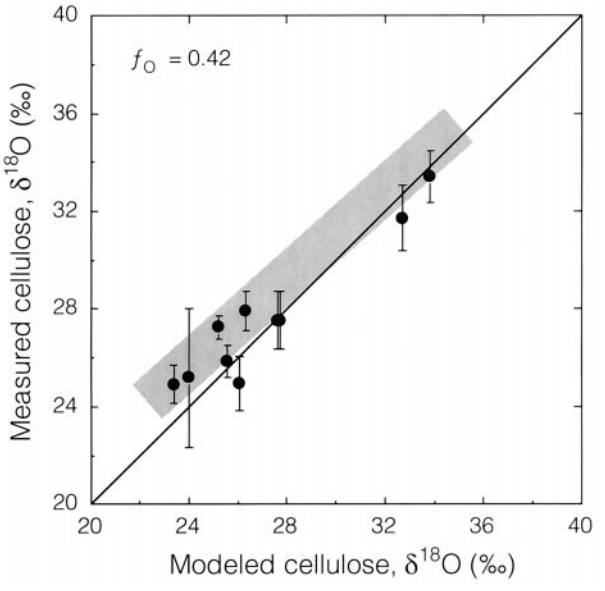

Fig. 9. The relationship between the $\delta^{18}O$ of modeled and measured tree-ring cellulose for field grown riparian zone trees. Variations in tree-ring cellulose were due to different stream water δD values and temperature and humidity differences between sites. Values are means and standard deviations. The line represents a 1:1 relationship. The gray band represents the predictions of the model using the measured range of leaf water values rather than the average leaf water value for a sampling date. From Roden and Ehleringer (1999c).

measurements of gas exchange parameters. The cellulose modeling was based on field observations of leaf water. Two modeling efforts were considered: (1) initially using the cellulose model to determine the best-fit f_O and f_H values to obtain the closest agreement between field cellulose observations and model predictions; and (2) secondarily constraining the cellulose model to use only the f_O and f_H values that had been derived from the greenhouse experiments. For oxygen isotope ratios in cellulose, there was a very strong agreement between the best-fit f_O value from field observations and well-controlled greenhouse experiments. Both observations predicted that f_O values should be 0.42. For the field observations, it is important to recognize that the leaf water values associated with cellulose synthesis may not have been exactly the same as those values collected at midday on a particular day of the month. Therefore, our modeling analyses considered the range of possible predictions based on the range of leaf waters observed rather than the average leaf water observed. The range of predicted cellulose isotope ratios is depicted in Figure 9 as the gray band. For hydrogen isotopes in field cellulose samples, the best-fit f_H value was 0.31 (Fig. 10), which is slightly lower than had been observed in the greenhouse experiment (0.36). When we constrain the model to use only the greenhouse observed f_H value of 0.36, the relationship between the model predictions and field observations is shown by the right-hand edge of the gray band in Figure 10. The left-hand edge of this gray band corresponds to the lower range of possible values, based on known field variations in leaf water values as previously described. It is important to recognize that the model is inherently more sensitive to variations in f_H values than in f_O values, because for oxygen the heterotrophic and autotrophic fractionation fac-

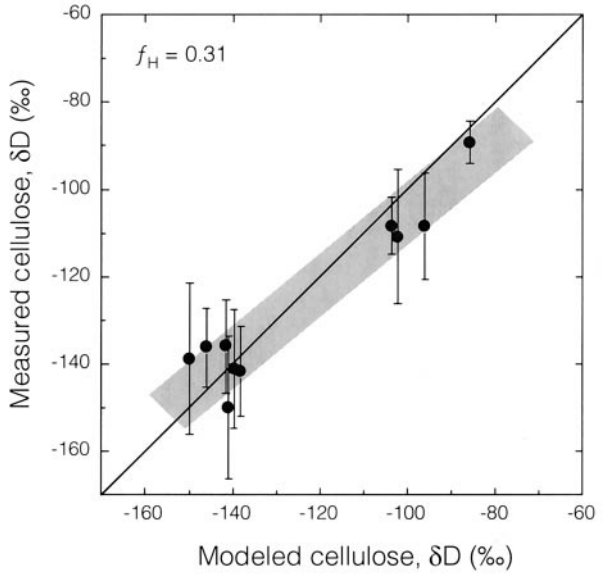

Fig. 10. The relationship between the δD of modeled and measured tree-ring cellulose for field grown riparian zone trees. Variations in tree-ring celulose were due to diferent stream water δD values and temperature and humidity differences between sites. Values are means and standard deviations. The line represents a 1:1 relationship. The gray band represents the predictions for cellulose isotope ratios if f_H was constrained to a value of 0.36 and if we include the observed range of leaf water values. From Roden and Ehleringer (1999c).

tors are identical, but they differ for hydrogen heterotrophic and autotrophic fractionation. The predicted differences in f_H values may be real or associated with the lack of environmental control in the field observations relative to the precise controls under greenhouse conditions. What factors might be the basis for possible variation in f_H values between greenhouse and field observations?

4.3. Triose Phosphate Recycling

The mechanistic basis for f_H and f_O values is in the metabolic pathways leading to cellulose synthesis. Theoretically 2 of the 10 oxygens in each cellobiose unit repeated in a cellulose molecule are expected to undergo exchange with medium water during synthesis from sucrose, leading to a potential f_O value of 0.2 (Farquhar et al., 1998). However, if a fraction of the hexose phosphates goes through the triose phosphate pathway, then 6 of 10 oxygen atoms become exchangeable. That is,

$$f_O = 0.6 \cdot y + 0.2, \quad (11)$$

where y is the fraction of hexose phosphates that are broken down into triose phosphates before being incorporated into cellulose. From results in Hill et al. (1995), Farquhar et al. (1998) calculated that ≈40 to 50% of the sucrose was broken down into triose phosphates before its incorporation into stem cellulose. Our data would indicate that y = 38%, which is indeed similar, and which may be viewed as ancillary evidence in support of similar f_O values for xylem cells during tree-ring cellulose synthesis. Our regression-based estimates of f_O values are similar to those reported by Yakir and DeNiro (1990) and Luo and Sternberg (1991, 1992).

The value for f_H was lower (0.36) than f_O, but still consistent with the conclusions of Luo and Sternberg (1992) who estimated from empiric relationships that f_H should be similar to f_O. The biochemical basis for exchange of hydrogen is less well documented than for oxygen; however, a similar analysis to Eqn. 11 should be appropriate. Although the C–H hydrogen is not exchangeable with medium water there are a number of opportunities for exchange during metabolism (Yakir, 1992). However, the greatest potential for H exchange with medium water occurs during triose phosphate isomerization and interconversion between the products fructose-6-P and glucose-6-P where half of the carbon bound hydrogens of each hexose could be exchanged (Yakir, 1992). For sugars that do not enter into the triose phosphate cycle, we estimate that 2 of 14 hydrogen in sucrose (that eventually become C–H bonded in cellulose) exchange with medium water if the isomerization between glucose-6-P and fructose-6-P is rapid. If we assume 50% exchange for hexose phosphates that go through triose phosphate cycling (Yakir, 1992) and then insert these different parameters for hydrogen into Eqn. 2, we estimate 43% triose phosphate cycling, which is close to the 38% estimated using oxygen.

Alternatively, invoking a futile cycle to explain f_H and f_O values may not be necessary (L. S. L. Sternberg, personal communication). If carbohydrates in general contain a similar portion of exchangeable hydrogen and oxygen atoms, then similar f_H and f_O values would be expected for starch, sucrose, and cellulose. At present, we are unable to distinguish between these two possible explanations for f_H and f_O. More work is needed to determine how much exchange occurs during sucrose synthesis and whether the same atoms are exchangeable during cellulose synthesis. In addition, estimates of triose phosphate cycling in various tissues and metabolic pathways would enhance our ability to predict f_H and f_O values for comparisons with experimental findings.

4.4. Current Model of Tree-ring Cellulose Is Fundamentally Different From Previous Models

We compared the predictions from our model of tree-ring cellulose isotope ratios with the most appropriate previous models: DeNiro and Cooper (1989), White et al. (1994), and Terwilliger and DeNiro (1995, although Terwilliger and DeNiro do not mathematically describe their model for stem cellulose, we assume that due to the assertion of isotopic reequilibration with xylem water that a 1:1 relationship between source water δD and tree-ring cellulose δD is predicted. All four models show similar predictions (a 1:1 relationship between cellulose and source water isotopic composition) under conditions where the difference between the δD values of source water and atmospheric water vapor are small (Fig. 11). However, when there is a departure in the isotope ratios of meteoric and atmospheric water, all four models predict different patterns (Fig. 12). Only our model correctly accounts for the observed cellulose isotope ratios derived from the greenhouse hydroponics study (Roden and Ehleringer, 1999a) under a wide range of environmental conditions.

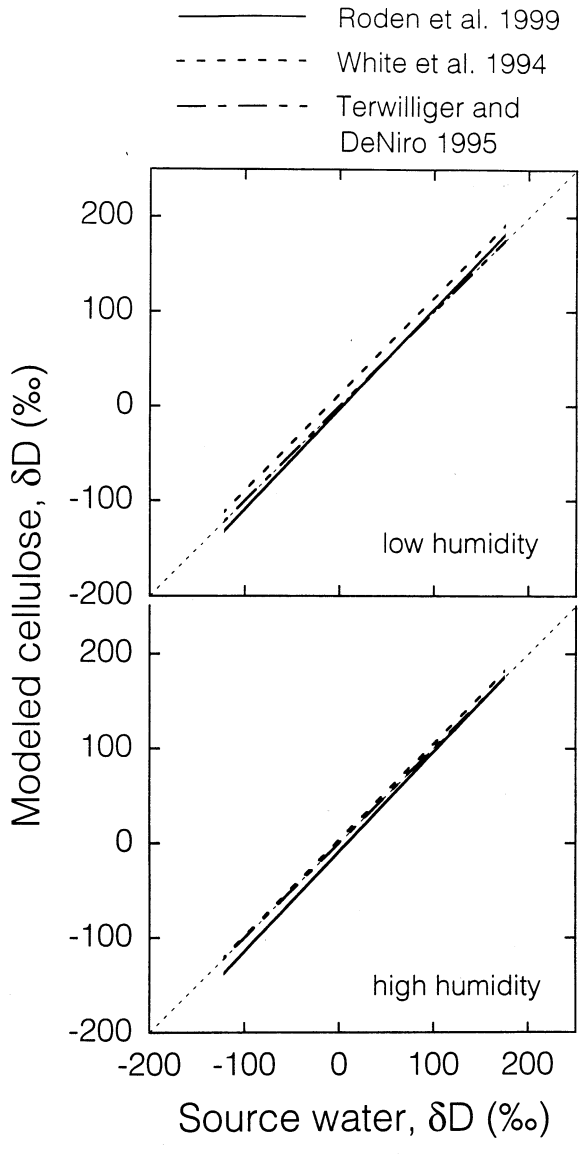

Fig. 11. Model comparisons for the estimates of δD in tree-ring cellulose if the atmospheric vapor δD was assumed to be in equilibrium with the water in each hydroponic tank (20‰ more negative than source water). Relative humidity and other environmental parameters were assumed to be identical to those measured or estimated in the greenhouse experimental system.

Our model clarifies some of the seemingly competing observations in the literature. Pioneering studies by Yapp, Epstein, and others (Epstein and Yapp, 1977; Burk and Stuiver, 1981; Yapp and Epstein, 1982; Lawrence and White, 1984; White et al., 1994) that suggested a 1:1 relationship between δD values of tree-ring cellulose and meteoric source water were correct in pointing out the importance of meteoric water in contributing to cellulose formation, but these investigators were unaware of the nearly offsetting hydrogen fractionation factors (ϵ_{HA} and ϵ_{HH}) involved in autotrophic and heterotrophic carbohydrate reactions. Later laboratory experimental studies of cellulose formed over a several-week period claimed that carbohydrates reequilibrate with medium water during cellulose synthesis for oxygen (DeNiro and Cooper, 1989) and hydrogen (Terwilliger and DeNiro, 1995) such that fractionation events in the leaf have little or no effect on tree-ring cellulose δD and $\delta^{18}O$ values. Yet the results of these studies were inconsistent with Yakir and DeNiro (1990), although it might be argued that leaf cellulose was investigated in one study whereas stem cellulose was investigated in the other two. Still in an experimental design in which xylem water and atmospheric water vapor were in near equilibrium to each other, the opposing signs and equivalent magnitudes of ϵ_{HH} and ϵ_{HA} could simply be masking some of the fractionation events taking place in the leaf and recorded in stem cellulose. Clearly, our results demonstrate that tree-ring cellulose can contain information related to atmospheric humidity and there is not complete exchange with medium water at the time of cellulose synthesis. Our model does agree with the predictions of Luo and Sternberg (1992) that $f_H \approx f_O$ and that $f_H \approx 0.34$ for cellulose derived from simple carbohydrates rather than lipids. We are also in near agreement with leaf cellulose studies of water ferns by Yakir and DeNiro (1990).

There is also an equally strong literature showing that δD values of tree-ring cellulose and relative humidity are linearly correlated (Yapp and Epstein, 1982; Edwards et al., 1985; Lipp et al., 1991). If we assume that leaf and air temperatures were similar and that the meteoric and atmospheric waters were reasonably constant during the growing period, then Eqn. 2 predicts that a strong humidity signal exists, which should be incorporated into photosynthetic products. Observations of only a partial isotopic exchange during subsequent cellulose formation within stem xylem provides the basis for previous humidity–isotope correlations. It is the diminished range of humidity values in some previous field studies that likely explains why such correlations were not seen in some studies. What we cannot explain is why previous laboratory studies by DeNiro and Cooper (1989) and Terwilliger and DeNiro (1995) did not detect an influence of humidity on stem cellulose isotope ratios.

4.5. Does the Proposed Model Satisfactorily Account for Previous Observations?

We believe that the proposed model satisfactorily explains the apparent conflict observed in the correlations of many previous tree-ring studies. Both meteoric water source and atmospheric humidity signals are incorporated into tree-ring cellulose. It is the limited range of humidity conditions and the limited differences in the isotope ratios of source water and atmospheric water that lead to the mistaken expectation that a humidity signal is not recorded in stem cellulose. The proposed model predicts that leaf and stem cellulose values should be different, because the source water pools are different. The model also predicts that stem cellulose and root cellulose should have the same hydrogen and oxygen isotope ratios, as the source of the carbohydrate substrate and the isotope ratios of the medium water would be the same. Roden and Ehleringer (unpublished data) confirmed both predictions under laboratory conditions.

Most studies that have focused on isotope–humidity signals contained in tree-rings have restricted analyses to that portion of the ring formed during the latter half of the growing season.

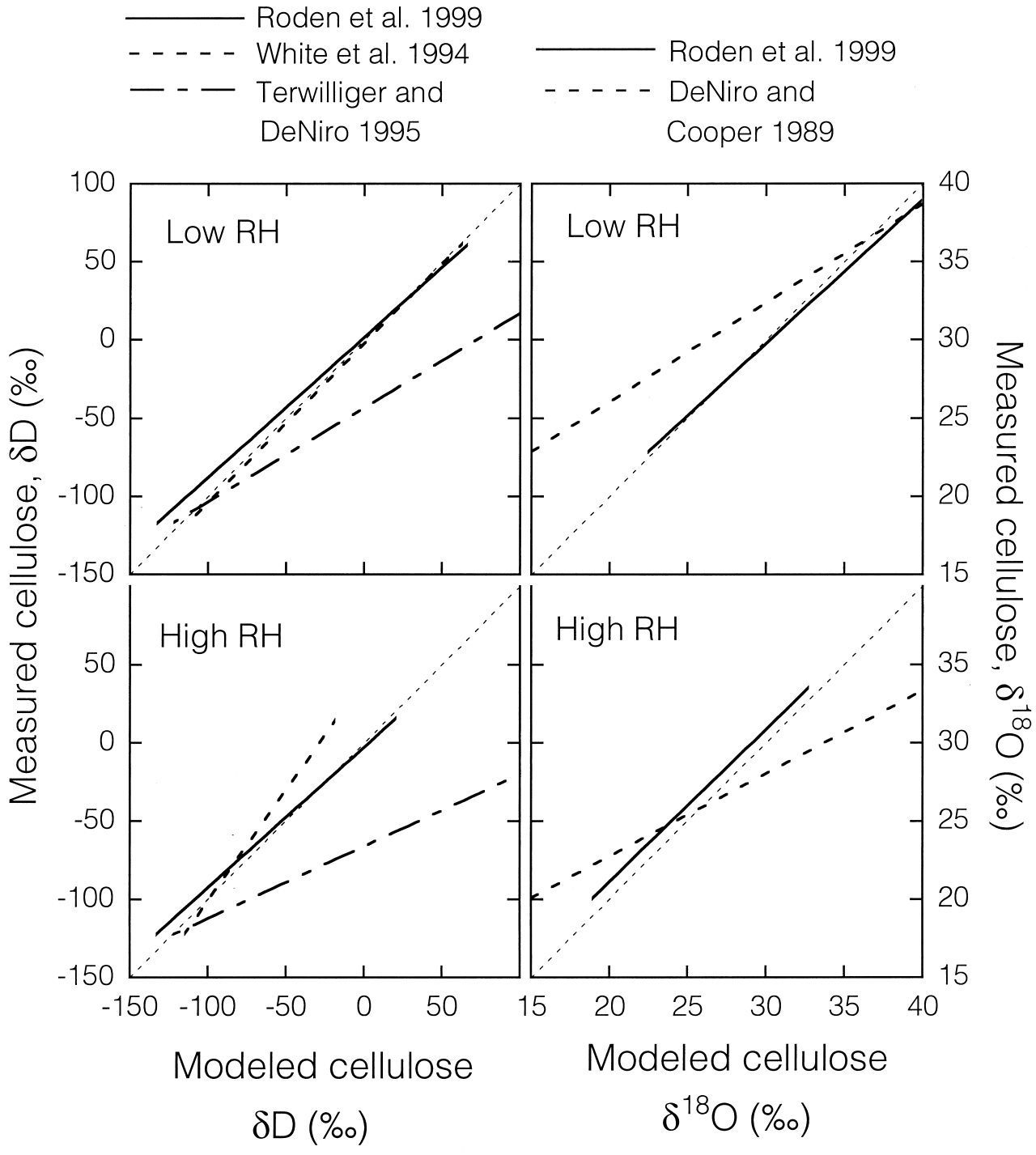

Fig. 12. Model comparisons for the estimates of δD and $\delta^{18}O$ in tree-ring cellulose for the environmental conditions measured or estimated in the greenhouse experimental system. Data are the modeled versus the measured values within the range of measured cellulose values from the greenhouse experiment. The fine dashed line represents the 1:1 relationship.

Often these studies observed no detectable patterns between cellulose isotope ratio and early season humidity conditions. It is expected that early-season cellulose formation in tree-rings will often be dependent on carbohydrates formed during a previous season, as photosynthetic rates are low or in some cases the stem xylem cells are produced before leaves have emerged. Therefore, tree-ring cellulose formed early in the year would not necessarily be expected to reflect source water and humidity conditions. In tree species with ring-porous xylem, such as oaks, these initial stem xylem cells are produced with water remaining in the stem after partial winter desiccation. Phillips and Ehleringer (1995) have shown that this stem water

can be very isotopically enriched by the end of the winter, differing by more than 45‰ and 15‰ for δD_{wx} and $\delta^{18}O_{wx}$, respectively, from the meteoric source water. During the growing season, δ_{wx} values do not deviate from the meteoric water line. Thus, hydrogen and oxygen isotope ratios of cellulose in early-season xylem of ring porous species should be interpreted with caution, as both the medium water and the substrate may be unrelated to either current meteoric source water or to current humidity conditions. The model presented here can also help clarify relationships between tree-ring hydrogen and oxygen isotope ratios and environmental parameters of interest to dendrologic or climate-reconstruction studies. However, because the isotope ratios of atmospheric water vapor can be influential in determining leaf water isotope ratios, assumptions on the relationships between source water and atmospheric water relationships should be clearly described. Where this factor may be most important is in those situations where precipitation falls primarily during the cooler winter months and growth occurs during warmer and drier spring–summer months. Such environmental conditions characterize many of the growth regimes of the conifers that have been used for isotope ratios studies within North America. Although the findings of this study complicates the straightforward interpretation of the δD signals in tree-ring cellulose as reflecting only precipitation inputs, it also means that those signals are richer in information. Much of the variation around the 1:1 relationship between source water and tree-ring cellulose in previous studies (as much as 25 to 50‰ in δD; Epstein and Yapp, 1977; Yapp and Epstein 1982) may not simply represent noise but rather additional environmental information such as humidity.

The results of experimental studies by Yakir and DeNiro (1990), Luo and Sternberg (1991, 1992), and Roden and Ehleringer (1999a, 1999b, 1999c) suggest common mechanisms influencing cellulose isotope ratios across a broad range of species. As shown by our proposed model and supporting experimental studies, the conclusion of Terwilliger and DeNiro (1995) that no universal formula exists linking source water and cellulose δD values is unjustified. Indeed, the opposite holds. There appears to be a predictable and common relationship for plants in which the hydrogen and oxygen isotope ratios of tree-ring cellulose record both source water and humidity conditions.

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APPENDIX 1

A. Listing of Variables Described in this Article

δD_{bulk}	hydrogen isotope ratio of bulk leaf water (‰, SMOW scale)
δD_{cx}	hydrogen isotope ratio of cellulose in tree-rings (‰, SMOW scale)
δD_{sp}	hydrogen isotope ratio of sugars transported in the phloem (‰, SMOW scale)
δD_{wl}	hydrogen isotope ratio of water in the leaves (‰, SMOW scale)
δD_{wv}	hydrogen isotope ratio of atmospheric water vapor (‰, SMOW scale)
δD_{wx}	hydrogen isotope ratio of water in the xylem of tree-rings (‰, SMOW scale)
$\delta^{18}O_{bulk}$	oxygen isotope ratio of bulk leaf water (‰, SMOW scale)
$\delta^{18}O_{cx}$	oxygen isotope ratio of cellulose in tree-rings (‰, SMOW scale)
$\delta^{18}O_{sp}$	oxygen isotope ratio of sugars transported in the phloem (‰, SMOW scale)
$\delta^{18}O_{wl}$	oxygen isotope ratio of water in the leaves (‰, SMOW scale)
$\delta^{18}O_{wv}$	oxygen isotope ratio of atmospheric water vapor (‰, SMOW scale)
$\delta^{18}O_{wx}$	oxygen isotope ratio of water in the xylem of tree-rings (‰, SMOW scale)
ϵ_{HA}	fractionation for hydrogen in autotrophic metabolism (dimensionless)
ϵ_{HH}	fractionation for hydrogen in heterotrophic metabolism (dimensionless)
ϵ_{O}	net biological fractionation for oxygen in going from sugars to cellulose (dimensionless)
α^*	water liquid-vapor equilibrium isotope effect, different for H and O (dimensionless)
α_k	isotope effect for water associated with diffusion in air, different for H and O (dimensionless)
α_{kb}	isotope effect for water associated with diffusion in the boundary layer, different for H and O
f_l	proportion of bulk leaf water subjected to evaporative enrichment (dimensionless)
f_H	proportion of carbohydrate δD signal from leaf carbohydrate that exchanges with local water during formation of tree-ring, leaf or root cellulose (dimensionless)
f_O	proportion of carbohydrate $\delta^{18}O$ signal from leaf carbohydrate that exchanges with local water during formation of tree-ring, leaf or root cellulose (dimensionless)
e	vapor pressure in bulk air (e_a), intercellular air spaces (e_i), or leaf surface (e_s) (kPa)
w	mole fraction of water vapor in bulk air (w_a), intercellular air spaces (w_i), or leaf surface (w_s) (dimensionless)
g_{sw}	stomatal conductance to water vapor (mol m^{-2} s^{-1})
E	transpiration rate (mmol m^{-2} s^{-1})

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