Oxygen-18 Content of Atmospheric Oxygen Does Not Affect the Oxygen Isotope Relationship between Environmental Water and Cellulose in a Submerged Aquatic Plant, *Egeria densa* Planch

Lee W. Cooper*2 and Michael J. DeNiro3

Department of Earth and Space Sciences, University of California, Los Angeles, California 90024

ABSTRACT

We determined that the oxygen isotopic composition of cellulose synthesized by a submerged plant, *Egeria densa* Planch., is related to the isotopic composition of environmental water by a linear function, \( \delta^{18}O \) cellulose \( = \) \( 0.48 \delta^{18}O \) water \( + 24.1\%o \). The observation of a slope of less than 1 indicates that a portion of cellulose oxygen is derived from an isotopically constant source other than water. We tested whether this source might be molecular oxygen by growing plants in the presence of high concentrations of \(^{18}O \) in the form of \( O_2 \) bubbled into the bottom of an aquarium. Cellulose synthesized during this experiment did not have significantly different oxygen isotope ratios than that synthesized by control plants exposed to \( O_2 \) of normal \(^{18}O \) abundance. We propose that oxygen in organic matter recycled from senescent portions of the plant is incorporated into cellulose. Our findings indicate that paleoclimatic models linking the oxygen isotopic composition of environmental water to cellulose from fossil plants will have to be modified to account for contributions of oxygen from this or other sources besides water.

Based upon experiments with \(^{18}O \)-labeled water, Ruben et al. (21) concluded that \( O_2 \) released during photosynthesis is derived from water. Additional experiments with \(^{18}O \)-labeled bicarbonate indicate that \( CO_2 \) does not contribute to the \( O_2 \) released (21), which has been taken by some as an indication that \( CO_2 \) is the source of oxygen incorporated into cellulose. Nevertheless a study using \(^{18}O \)-labeled \( CO_2 \) indicated that the source of oxygen in cellulose synthesized by wheat plants cannot be \( CO_2 \) (6). This study demonstrated that an enormous change in the oxygen isotopic composition of \( CO_2 \) supplied to wheat plants results in only a marginal change in the oxygen isotopic composition of the cellulose synthesized. DeNiro and Epstein (6, 7) reconciled this apparent contradiction with the experiments conducted by Ruben et al. (21) by proposing that complete isotopic exchange during reactions leading to cellulose synthesis occurs between the oxygen of water and those derived from \( CO_2 \). This causes the loss of the characteristic O isotope signature contributed by \( CO_2 \).

This conclusion is important for paleoclimatic studies because it implies that the O isotope composition of cellulose is directly related to leaf water O isotope composition (3, 8). If additional factors that influence cellulose O isotope ratios can be quantified, particularly biochemically induced isotopic fractionations between leaf water and cellulose O and the \(^{18}O \)-enrichments in leaf water that occur during evapotranspiration (reviewed by Sternberg [26]), accurate paleoclimatic reconstructions should be possible from the analysis of \(^{18}O/^{16}O \) ratios of cellulose in fossil plants. These paleoclimatic models would use the O isotopic composition of cellulose and all relevant isotopic fractionation processes to reconstruct the probable isotopic composition of water available to the plant, which is related to climatic parameters (5, 16).

Empirical models have been developed that account for the relationship between O isotope ratios of environmental water and cellulose (3, 8). However, one of the major assumptions made, that the only source of O that affects the \(^{18}O/^{16}O \) ratios of cellulose if leaf water, may not be true. The evidence concerning the validity of this assumption comes from submerged aquatic plants, which can be used advantageously in studies of the relationship between O isotope composition of water and cellulose. Due to the lack of transpiration, the \(^{18}O/^{16}O \) ratio of tissue water in submerged plants is close to that of the water in which the plant grows (2). This is not the case for terrestrial plants or emergent aquatic plants, which can have leaf water significantly enriched in the heavy isotopes of O and H relative to source water due to isotopic effects during evapotranspiration (2, 11, 12, 17, 19, 29). Because there is no evapotranspiration in submerged plants, only isotope effects associated with biochemical reactions influence the oxygen isotopic composition of cellulose. If, for any individual submerged species, these isotope effects are constant for specimens grown under identical conditions, and if water is the only source of oxygen that influences the \(^{18}O/^{16}O \) ratios of cellulose, then the oxygen isotope composition of cellulose of that species should vary in a one-to-one fashion with the oxygen isotope composition of water in which the plants grew (11).

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2 Present address: Department of Geological Sciences, University of Tennessee, Knoxville, TN 37996-1410 and Environmental Sciences Division, Oak Ridge National Laboratory, P. O. Box 2008, Oak Ridge, TN 37831-6038.

3 Present address: Department of Earth Sciences, University of California, Santa Barbara, CA 93106.

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Ferhi et al. (14) did not find a one-to-one relationship between the oxygen isotope composition of environmental water and the oxygen isotope composition of cellulose in their field study of submerged aquatic plants. In their work, Ferhi et al. determined the following relationship:

$$\delta^{18}O_{\text{cellulose}} = 0.65 \delta^{18}O_{\text{ambient water}} + 25 \cdot 4\%o$$  

(1)

where

$$\delta^{18}O = (\frac{\delta^{18}O_{\text{sample}}/\delta^{18}O_{\text{SMOW}} - 1}{} \times 1000\%o$$  

(2)*

The less than one-to-one relationship suggests that, in addition to water, a form of oxygen with a constant isotopic composition affects the isotopic composition of cellulose. Ferhi and Letolle (13), based on work with terrestrial plants, had already suggested that molecular atmospheric oxygen ($\delta^{18}O = +23.5\%o$), incorporated through photorespiration, and carbon dioxide ($\delta^{18}O = +41\%o$), provide oxygen that is incorporated into cellulose. The work of DeNiro and Epstein (6), apparently excludes the possibility of significant CO$_2$ contributions. Tracer studies using $^{18}$O have shown that oxygen from O$_2$ can be incorporated into metabolic cycles through photorespiration (1), although even the earliest work with stable oxygen isotope ratios in plants suggests that this contribution is not substantial (21). This unresolved question led to the current study in which we grew a submerged plant, Egeria densa Planch., in an aquarium tank exposed to high concentrations of $^{18}$O$_2$ to test whether the oxygen isotope composition of newly synthesized cellulose was affected.

As an initial step, however, we determined in a more controlled manner the oxygen isotope relationship between cellulose and environmental water in E. densa. The study of Ferhi et al. (14) that generated Equation 1 can be improved upon in several ways. First, it was a field study in which the oxygen isotope composition of the water available to plants was not sampled continuously, was not known precisely, and could not be experimentally controlled. Second, since the work involves interspecific comparisons, there is the possibility that biochemically induced isotopic fractionations in the nine species that were studied may be different, thus affecting the cellulose-water isotopic relationship. Finally, $\delta^{18}O$ values for intracontinental waters range from $-50$ to $+30\%o$ (15). The range of $\delta^{18}O$ values in waters in which plants grow is somewhat smaller, but nevertheless considerably larger than Ferhi et al. (14) were able to sample ($\delta^{18}O$ values from $-12.7$ to $+8.1\%o$).

Thus, we decided to grow E. densa in separate aquarium with water of isotopic composition roughly spanning that in which plants can be found. The goal of this portion of the study was to determine if the oxygen isotope relationship between environmental water and cellulose varies in a one-to-one fashion in at least this one submerged species and to quantify the regression equation more accurately than Ferhi et al. (14) may have been able to. In the second phase of the study we examined the influence of molecular oxygen upon cellulose $\delta^{18}O$ values.

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*Abbreviation: SMOW, standard mean ocean water.

**MATERIALS AND METHODS**

In the first phase of the study, Egeria densa Planch. plants were grown under identical conditions except for the $^{18}$O/$^{16}$O ratios of the water they were supplied. Individual plant sprigs, each consisting of approximately 10 g wet weight, were rooted in five adjoining covered aquaria (No. 1–5 in Table 1) containing 25 L of water at constant temperature (22 ± 2°C) from August 18, 1987 to September 30, 1987. Continuous lighting was supplied by plant grow lights (Sylvania Gro-Lux) and a nearby sunlit window. To decrease evaporation, the sole egress for air bubbled into the bottom of the aquaria was through ice-cooled coils, which condensed water vapor and returned liquid water to the aquaria. The waters used were initially distilled from three isotopically distinct sources: snow from the summit of Ester Dome, Fairbanks North Star Borough, AK ($\delta^{18}O = -22.4\%o$); University of California, Los Angeles (UCLA) tap water ($\delta^{18}O = -11.4\%o$); and University of California, Santa Barbara (UCSB) tap water ($\delta^{18}O = -3.9\%o$).

Two additional waters with $\delta^{18}O$ values of $+14.1\%o$ and $+21.3\%o$ were made by adding appropriate volumes of water with 1.5 atom % $^{18}$O (MSD Isotopes, Montréal, Québec) to the UCSB water. To each aquarium containing distilled water, 10 mL of trace element solution (Tetra Flora Pride) and 5 mL of liquid plant fertilizer (Gro-Power 4-8-2, Southern California Organic Fertilizer Co., El Monte, CA) were added. In addition, 250 mL of water was added, as a conditioner, to each of the five aquaria from an aquarium in which E. densa plants were being grown before the start of the study. Oxygen isotopic composition of the water in each aquarium was determined, at a minimum, at the beginning and the end of each growth period. At the end of the first experiment, new plant growth was harvested from each aquarium and freeze-dried prior to cellulose extraction.

In the second experiment, E. densa plants were grown under identical conditions except for the $\delta^{18}O$ values of the O$_2$ they were supplied. The plants were grown in two adjoining covered aquaria from June 27 to July 21, 1988. The lighting, ice-cooled condenser system, water, and so forth were as in the first experiment, except the distilled water introduced into each aquarium came from a common source ($\delta^{18}O = -16.6\%o$). The oxygen isotope content of the waters were determined on a weekly basis for each aquarium. A single compressed air tank was used to bubble air into the bottom of each aquarium at a rate of 200 mL/min (ambient pressure and temperature). Two oxygen tanks supplied an additional 2 mL of O$_2$/min to the air bubbling into each aquarium. Oxygen from these tanks was injected directly into the air through glass hypodermic needles that had been sealed with Apiezon W wax into the two airstream hoses. The control oxygen tank was obtained locally and contained natural abundances of $^{16}$O. The experimental oxygen tank, (custom mixture, Isotec, Inc., Miamisburg, OH) contained approximately 1.05 atom % $^{18}$O labeled O$_2$ (approximate $\delta^{18}O = +1600\%o$).

Because of the possibility of contaminating the mass spectrometer used, no attempt was made to measure the isotopic composition of the $^{18}$O-enriched oxygen tank. Instead, air-oxygen samples were taken from the air-oxygen stream directly before it entered each of the two aquaria on four
separate occasions, with replicate samples taken each time. The gas sampling vessels were approximately 15 mL in volume and contained a greased stopcock at each end. The vessels were connected to the hoses delivering the air-oxygen flow stream to the aquaria. The air-oxygen mixture was allowed to flow through the sampling vessels for 10 min after which the two stopcocks were closed simultaneously. The oxygen isotope composition of O₂ in each sampling vessel was determined in the following manner (10). Trace quantities of H₂O and CO₂ were removed by passing the air-oxygen mixture through a liquid nitrogen trap. Oxygen was then converted to CO₂ by circulating the contents of each vessel over hot graphite using a Toepler pump. The CO₂ was condensed in a liquid nitrogen trap as it formed. After conversion to CO₂ was complete, the volumes of CO₂ and the nitrogen-argon mixture that remained after O₂ conversion were determined manometrically. The δ¹⁸O value of the CO₂ was then measured mass spectrometrically.

Carbon dioxide in the air-oxygen mixture flowing into each aquarium was sampled in a similar manner, but using a 1 L sampling vessel with greased stopcocks at either end. After remaining in the airstream for at least 1 h, both stopcocks were closed. Carbon dioxide was separated by slow passage of the vessel contents through two liquid nitrogen traps on a vacuum manifold over 1 h. Water was separated from the CO₂ cryogenically. The δ¹⁸O value of the CO₂ was then determined.

Plants were collected for cellulose extraction and oxygen isotope determinations at the end of the first experiment and at both the beginning and end of the second experiment. Only growth arising from new buds or nodes was harvested at the ends of the experiments. Each plant sample consisted of approximately 1 g wet weight. The following methods (11) were used for oxygen isotope determination of cellulose. Cellulose was extracted using a sodium chlorite-acetic acid oxidation procedure (30). Cellulose oxygen isotope ratios were determined by pyrolyzing vacuum-dried and sealed samples in the presence of HgCl₂ at 520°C for 5 h to form CO, CO₂, and HCl. CO was disproportionated to CO₂ and C by electrical discharge. HCl was removed by reaction with isoquinoline (11). The CO₂ was then analyzed mass spectrometrically.

Oxygen isotope ratios of water samples were determined by equilibrating 1.0 mL water samples with approximately 300 μmol of carbon dioxide for 48 h, purifying the equilibrated carbon dioxide cryogenically, analyzing the CO₂ mass spectrometrically, and using mass balance considerations to calculate the original oxygen isotope composition of the water (9).

Precisions of δ¹⁸O values determined for cellulose and water were ±0.5‰ and ±0.1‰, respectively.

RESULTS

No evidence was seen that the oxygen isotope composition of waters in any aquaria changed significantly over the course of either experiment (Table I). The results of the first experiment indicate that oxygen isotope ratios of cellulose in Egeria densa are related linearly to environmental water, but not in a one-to-one fashion (Fig. 1):

Table I. Oxygen Isotopic Composition of Water in Aquaria Over Course of First (Aquaria 1–5) and Second (Aquaria 6, 7) Experiments

<table>
<thead>
<tr>
<th>Aquarium</th>
<th>δ¹⁸O ± sd (%o)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>−22.3 ± 0.1</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>−11.2 ± 0.2</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>−3.9 ± 0.1</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>+14.0 ± 0.2</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>+21.3 ± 0.1</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>−16.6 ± 0.1</td>
<td>4</td>
</tr>
<tr>
<td>7</td>
<td>−16.6 ± 0.3</td>
<td>3</td>
</tr>
</tbody>
</table>

Figure 1. Relationship between oxygen isotope ratios of water and cellulose in aquatic plants synthesized by E. densa. Darkened circles correspond to Aquaria 1–6, which were supplied with O₂ of normal ¹⁸O abundance. The clear circle corresponds to Aquaria 7, which was supplied with ¹⁸O-enriched O₂. The circumscribed circle corresponds to Aquaria 6, which was the control for Aquaria 7. Regression line corresponds to Equations 3 and 5. SMOW, standard mean ocean water.

Table II. Oxygen Isotopic Composition of O₂ and CO₂ Entering Aquaria during Experiment

<table>
<thead>
<tr>
<th>Aquarium</th>
<th>O₂</th>
<th>CO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>δ¹⁸O ± sd</td>
<td>n</td>
</tr>
<tr>
<td>6</td>
<td>+26.1 ± 0.8</td>
<td>8</td>
</tr>
<tr>
<td>7</td>
<td>+33.8 ± 53.6</td>
<td>8</td>
</tr>
</tbody>
</table>

*The difference between experimental and control CO₂ isotopic composition may be due to oxidation of traces of organic carbon in the two oxygen tanks by oxygen.

\[
\delta^{18}O_{\text{cellulose}} = 0.48 \delta^{18}O_{\text{water}} + 24.1\%o; \quad r^2 = 0.98; \quad n = 7; \quad 0.001 < P < 0.005
\]

Results of air-oxygen sampling during the second experiment indicate that the gases flowing into aquaria 6 and 7 contained 22.00 ± 0.05% O₂, which, as expected, is slightly higher than atmospheric proportions of oxygen (21%). High concentrations of ¹⁸O-labeled oxygen gas were present in the air bubbled into aquarium 7 (Table II). Analysis of cellulose collected from plants at the start and end of the experiment indicated that the ¹⁸O content of cellulose synthesized in both aquaria 6 and 7 changed significantly from its initial value (Table III). The magnitude of isotopic shift was similar in both aquaria, however, and no evidence was seen that elevated ¹⁸O concentrations in molecular oxygen had any effect on the
Table III. Oxygen Isotopic Composition of Cellulose Synthesized by *E. densa* during Second Experiment

<table>
<thead>
<tr>
<th>Aquarium</th>
<th>δ18O (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 (normal abundance O₂)</td>
<td>+16.4</td>
</tr>
<tr>
<td>7 (18O-enriched O₂)</td>
<td>+15.5 +15.5</td>
</tr>
<tr>
<td>Initial, June 28, 1988</td>
<td>+23.2 ± 0.6 sp (n = 5)</td>
</tr>
</tbody>
</table>

Oxygen isotope composition of cellulose synthesized. When the data from plants collected at the end of the second experiment are combined with those of the first (Fig. 1), the following relation is obtained:

\[
\delta^{18}O_{\text{cellulose}} = 0.49 \delta^{18}O_{\text{water}} + 24.1 \%o; \quad r^2 = 0.98; \quad n = 10; \quad P < 0.001
\]

**DISCUSSION**

The results presented in this study are not consistent with any current model relating the oxygen isotopic composition of plant cellulose to environmental water. There are three models: (a) carbon dioxide, in isotopic equilibrium with water, contributes two-thirds of the oxygen to cellulose with the remaining one-third provided by environmental water (11); (b) oxygen isotope ratios in cellulose are determined by isotopic equilibration during carbonyl hydration reactions of some of the intermediates leading to cellulose synthesis (7); (c) oxygen isotope ratios of cellulose are determined by mixing oxygen of relatively constant isotopic composition from dissolved oxygen and/or carbon dioxide with oxygen from waters in which 18O/16O ratios vary as a function of environmental climate (13).

The first model is already in some disfavor because there is no evidence for temperature effects upon oxygen isotope fractionation between cellulose and water, as would be expected if carbon dioxide equilibrates with water before photosynthesis (7). Furthermore, the model cannot account for the observation that the cellulose-water δ18O relationship is the same for tunicates, which produce cellulose nonphotosynthetically, as it is for submerged aquatic plants (7). This model also specifically predicts a one-to-one relationship between cellulose δ18O values and water δ18O values for aquatic plants (11) and is thus at variance with our results. The second model (the carbonyl exchange hypothesis) requires a constant 27% enrichment during equilibration of carbonyl oxygens with water (7, 25). Cellulose δ18O values in this study were higher than those of environmental water by 14 to 36% (Fig. 1). The results reported here for *Egeria densa* are consistent with the third model, which involves mixing of oxygen from water with oxygen from another source with a constant 18O/16O ratio. The present study and that of DeNiro and Epstein (6) indicate, contrary to the model of Ferhi and Letolle (13), that neither O₂ nor CO₂ has any influence on the 18O/16O ratios of cellulose.

A possible explanation for the absence of a one-to-one relationship between the δ18O values of cellulose and water for *E. densa* comes from a consideration of its natural history, which suggests that new growth is partly dependent on translocation of organic material from senescent portions of the plant. Although native of a small area at the boundaries of Uruguay, Brazil, and Argentina, it is now distributed throughout large areas of North America, Europe, Africa, New Zealand, and Asia. Any detached fragment, if it includes a bud or node, can regenerate a new individual. Although dioecious, the female flowers are rare and all invasive populations are thought to be male (22). New healthy growth was readily generated in this study even from senescent plants. Thus, the oxygen isotope relationship between environmental water and cellulose for *E. densa* may not be a function only of the water isotopic composition, but also a function of the extent of organic material recycling and oxygen isotope effects during that process.

Studies using suspension cell cultures indicate that if metabolic intermediates (e.g. glucose, glycerol) are provided to cells, less than one-to-one relationships between the oxygen isotope composition of cellulose and water in which the cells grew are observed. This indicates that less than complete exchange between oxygen derived from intermediates and water occurs (25). DeNiro and Cooper (unpublished data) found that the oxygen isotopic composition of cellulose in potato (*Solanum tuberosum* L.) shoots, whether sprouted in the dark or light, is influenced primarily by the oxygen isotopic composition of the tuber water, not that of the potato starch that serves as the carbohydrate source for the shoot cellulose. In this case, complete, or nearly complete exchange between the oxygens of tuber starch and tissue water occurs by the time cellulose is synthesized in the shoot. In contrast, sprouting shoots from castor bean (*Ricinus communis* L.) seeds do not reflect the isotopic composition of water in which they are germinated on a one-to-one basis (25). Thus, when oxygen sources other than CO₂, such as organic matter present in seeds or storage tissue, are available to plants for cellulose synthesis, the extent of oxygen isotopic exchange between water and the organic substrate will depend on the specific plant substrate and pathways involved in cellulose synthesis.

We propose that the less than one-to-one correlation between the δ18O values of water and *E. densa* cellulose (Eqs. 3 and 4) results from incorporation of oxygen into cellulose from metabolic intermediates more reduced than CO₂. These recycled metabolic intermediates arise from organic matter within the plants that were introduced into the aquaria containing waters of different δ18O values. As a result, the δ18O values of these intermediates are unrelated to those of the environmental water used for cellulose synthesis during growth in the aquaria. Oxygen from these intermediates apparently does not undergo complete exchange with water in the course of cellulose synthesis in *E. densa*. If this explanation is correct, we allowed a longer period of growth prior to harvest, the slope of the regression line between the δ18O values of water and cellulose might have reached unity as the fraction of oxygen derived from water in the new biomass became so large as to overwhelm the contribution of oxygen from the original biomass.

These conclusions have obvious implications for experimental design in studies of the relationship between δ18O values of water and cellulose, but they are also relevant to
field studies of submerged aquatic plants. When we plotted the data from all previous field studies reporting $\delta^{18}O$ values for cellulose from submerged aquatic plants and environmental water (Fig. 2), we obtained the relationship given by the regression equation:

$$\delta^{18}O_{\text{cellulose}} = 0.78 \delta^{18}O_{\text{ambient water}} + 26.2^{0/o};$$

$$r^2 = 0.88; \quad n = 56; \quad P < 0.001$$

The standard error associated with this slope is ±0.04. Thus, natural populations of aquatic plants do not exhibit a one-to-one relationship between the $\delta^{18}O$ values of cellulose and water. It is unlikely, however, that the explanation we proposed for observations of *E. densa* grown in the aquaria can account for the observation reported in Equation 5. Organic intermediates in the aquatic plants that grew in the field under the variety of climatic and environmental regimes presented in Figure 2 would not have a single $\delta^{18}O$ value, as we proposed in our model for *E. densa*. Instead, in each case, the $\delta^{18}O$ value of the intermediates would be related to the $\delta^{18}O$ value of environmental water. Thus, some other mechanism must be responsible for the deviation from a slope of one seen in Figure 2 and Equation 5. Our results suggest it cannot be incorporation of oxygen from atmospheric $O_2$. Accordingly, we propose that the influence of the $\delta^{18}O$ value of ambient CO$_2$ should be reexamined.

Although Epstein et al. (11) asserted that dissolved CO$_2$ used in aquatic plant photosynthesis must be in isotopic equilibrium with water, this condition may not be consistently satisfied, because of the evidence for inorganic carbon uptake and recycling from CO$_2$ stored within the lacunae, or gas spaces, found within most aquatic vascular plants (4, 18, 20, 23). Although the presence of carbonic anhydrase should facilitate a rapid attainment of equilibrium between the oxygens of water and the oxygens of the dissolved carbon dioxide system, it is not known if the gas phase CO$_2$ in aquatic plant lacunae is in isotopic equilibrium with environmental water. Given this alternate route for providing inorganic carbon to the site of photosynthesis, and the importance of uptake of lacuna-derived gas phase carbon dioxide in many aquatic plants (4, 18, 20, 23), we have divided the data provided by previous workers (Fig. 2) into two groups, which correspond to plants with and without lacunae. Lacunae are derived from xylem tissue (28), so algae, mosses and other nonvascular plants lack these structures. The oxygen isotope compositions of cellulose are related to environmental water by the following relations for plants with (Equation 6) and without (Equation 7) lacunae:

$$\delta^{18}O_{\text{cellulose}} = 0.80 \delta^{18}O_{\text{ambient water}} + 26.5^{0/o};$$

$$r^2 = 0.86; \quad n = 36; \quad P < 0.001$$

$$\delta^{18}O_{\text{cellulose}} = 0.92 \delta^{18}O_{\text{ambient water}} + 26.0^{0/o};$$

$$r^2 = 0.90; \quad n = 20; \quad P < 0.001$$

The standard errors associated with the slopes of Equations 6 and 7 are ±0.06 and ±0.07, respectively. The uncertainty associated with Equation 7 is probably larger because there are few data available for nonvascular plants growing in waters that are relatively $^{18}$O-depleted (Fig. 2). The slope of Equation 7 is highly dependent on a single lake water measurement ($\delta^{18}O = -10.9^{0/o}$) at Siesta Lake, CA. (24). If this measurement of lake water oxygen isotope composition is not representative of the water available at the time of cellulose synthesis, Equation 7 is not accurate. The slope in Equation 6 is more certain because $\delta^{18}$O values from several other lakes relatively depleted in $^{18}$O, in addition to Siesta Lake, are available. Nevertheless, the apparent difference in slope between vascular and nonvascular plants suggests that the relationship between the oxygen isotope composition of cellulose and the oxygen isotope composition of water may be different in vascular and nonvascular plants. The apparent difference is not large, but additional data on the oxygen isotope composition of cellulose in nonvascular plants growing at high latitudes and/or altitudes would clarify whether there is a one-to-one relationship between $\delta^{18}$O values of water and cellulose in plants lacking lacunae. If such plants display a one-to-one relationship, the slope of less than one for plants with lacunae (Eq. 6) may be due to the absence of isotopic equilibrium between water and CO$_2$ stored in and taken up through lacunae. Another test of this possibility would be to determine if submerged vascular plants that lack lacunae, such as the Podostemaceae (22), exhibit one-to-one relationships between the $\delta^{18}$O values of environmental water and cellulose.

For terrestrial plants, it is difficult to determine if oxygen sources other than water influence the $\delta^{18}$O values of cellulose. Evapotranspiration causes the $\delta^{18}$O values of leaf water to vary diurnally and seasonally by so much that plots such as Figure 2 cannot be made for terrestrial plants. We note, however, that studies of bean plants (*Phaseolus vulgaris* L.) indicate that cellulose $\delta^{18}$O values do not bear a one-to-one relationship with those of leaf water, even after variable humidity factors that influence leaf water isotope ratios have been taken into account (14). Thus, some other source of oxygen besides H$_2$O and equilibrated CO$_2$ must also affect terrestrial plant cellulose $\delta^{18}$O values. If our results can be extended to terrestrial plants, this source cannot be molecular oxygen.
These considerations have important consequences for the use of cellulose δ18O values to characterize the oxygen isotopic composition of environmental water, such as in paleoclimatic studies. Qualitative and quantitative interpretations of fossil plant cellulose isotope ratios from this perspective have been based on the assumption that the only source of oxygen that affects the δ18O value of cellulose is leaf water. The results reported here and by others (Fig. 2) indicate this assumption is not valid. The identity of other oxygen sources whose 18O/16O ratios also affect those of cellulose must be established before paleowater δ18O values are reconstructed from fossil cellulose oxygen isotopic compositions.

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LITERATURE CITED