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Identifying the $\delta^{18}O$ signature of precipitation in grass cellulose and phytoliths: Refining the paleoclimate model

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Abstract

Cellulose and silica phytoliths were extracted from the leaves and stems of *Calamovilfa longifolia*, a C_4 grass, grown under varying climatic conditions across the North American prairies. The oxygen-isotope compositions of both cellulose and silica record a complex signal of the isotopic composition of the soil water that feeds the plants and the relative humidity conditions that influence transpiration rates, stomatal conductance, and ultimately the ^{18}O-enrichment of leaf water. As the initial stages of cellulose formation occur in the leaves, cellulose in both the leaves and stems forms primarily from leaf water and does not differ greatly in its oxygen-isotope composition between these locations. In contrast, the $\delta^{18}O$ values of leaf phytoliths are significantly enriched in ^{18}O relative to stem phytoliths, reflecting the varying isotopic composition of the water in these tissues. The oxygen-isotope compositions of leaf cellulose may be used as a proxy for the isotopic composition of water involved in leaf phytolith formation, while the $\delta^{18}O$ values of stem phytoliths can be used to determine the $\delta^{18}O$ values of stem water involved in partial exchange reactions during the transport of carbohydrates through the plant. A comparison of the isotopic compositions of phytoliths with cellulose allows for the deduction of soil and leaf water $\delta^{18}O$ values as well as temperature and relative humidity conditions during plant growth. This approach has application in paleoclimate studies that traditionally have required estimations of one or more of these variables because direct measurements were unavailable.

1. Introduction

The oxygen-isotope composition of plant cellulose is determined by the $\delta^{18}O$ values of water feeding the plant, modification to leaf water $\delta^{18}O$ values during transpiration, and biochemical pathways within the plant. Hence, the $\delta^{18}O$ values of cellulose can be used as a proxy for the temperature and relative humidity that influenced transpiration during plant growth. The basis for using cellulose $\delta^{18}O$ values as a quantitative temperature proxy is a reliable correlation between temperature and the $\delta^{18}O$ values of precipitation feeding the plant (e.g., Epstein et al., 1977). However, the oxygen-isotope composition of precipitation can be modified prior to incorporation into cellulose via mixing and evaporation in the soil (Allison et al., 1984; Tang and Feng, 2001; Gazis and Feng, 2004), leaf water ^{18}O enrichment during transpiration (Webb and Longstaffe, 2003), and oxygen-isotope exchange between cellulose precursors and stem and/or leaf waters (Farquhar et al., 1998).

Biogenic silica bodies (phytoliths) formed in the cells of terrestrial plants are also a ubiquitous, well-preserved component of most soils (Fredlund and Tieszen, 1997). Quantitative paleoclimate information can be obtained through the isotopic analysis of phytoliths. Their $\delta^{18}O$ values are determined by growth temperature and the isotopic composition of plant water, which in turn are dependent on the $\delta^{18}O$ value of precipitation feeding the plants and climate conditions that influence transpiration rates (Shahack-Gross et al., 1996; Webb and Longstaffe, 2000). Hence, for both the cellulose and phytolith oxygen-isotope systems, paleoclimate interpretations (i.e., temperature, relative humidity) are limited by our ability to estimate ancient plant water $\delta^{18}O$ values. These compositions can

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fluctuate on a seasonal and daily basis and even differ between tissues of the same plant. However, when the $\delta^{18}O$ values of phytoliths and cellulose are used together, it should be possible to approximate the $\delta^{18}O$ values of soil and leaf water over the growing season.

The oxygen-isotope composition of water within a plant remains unchanged from that of soil water until affected by loss of water via transpiration in the leaves, which results in the residual leaf water being enriched in ^{18}O. The degree of ^{18}O enrichment is inversely related to relative humidity (h) and can be modelled by the Craig and Gordon (1965) equation for evaporation from a standing body of water, as modified by Dongmann et al. (1974) to describe evaporation from plant leaves:

$$\delta^{18}O_{\text{modelled leaf water}} = \delta^{18}O_{\text{stem water}} + \varepsilon_k + \varepsilon^* + h(\delta^{18}O_{\text{atm}} - \varepsilon_k - \delta^{18}O_{\text{stem water}}), \quad (1)$$

where $\varepsilon^* = (\alpha - 1) \times 1000$ is the equilibrium fractionation factor (Majoube, 1971), $\delta^{18}O_{\text{atm}}$ is the $\delta^{18}O$ value of atmospheric water vapour and ε_k is the kinetic fractionation factor (Merlivat, 1978; Buhay et al., 1996; Cappa et al., 2003). There is a strong gradient in $\delta^{18}O$ values between water at the sites of evaporation ($\delta^{18}O_{\text{modelled leaf water}}$) and incoming vein water ($\delta^{18}O_{\text{stem water}}$) caused by diffusion of heavy isotopes away from the sites of evaporation (the Péclet effect) (Farquhar and Lloyd, 1993). Hence, if a large amount of stem water is required to replenish leaf waters when transpiration rates are high, bulk leaf water $\delta^{18}O$ values may be lower than $\delta^{18}O_{\text{modelled leaf water}}$ values. In addition, the parallel venation of grass leaves allows mixing between ^{18}O-enriched leaf water and vein waters as they move towards the tip of the leaf resulting in their overall ^{18}O enrichment from base to tip of the leaf (Helliker and Ehleringer, 2000). Such progressive enrichment can produce $\delta^{18}O$ values of bulk leaf water that are higher than the $\delta^{18}O_{\text{modelled leaf water}}$ values predicted by Eq. (1). In grasses, the stem water $\delta^{18}O$ value generally approximates the weighted average $\delta^{18}O$ value of soil water over the plant's rooting depth (Webb and Longstaffe, 2003).

Grasses take up aqueous silicic acid passively with soil water and become silica-saturated though the removal of water via transpiration (Kaufman et al., 1981; Perry and Mann, 1989). Silica is then precipitated as opal-A, within and between plant cells in close proximity to the transpiration stream. The $\delta^{18}O$ values of phytoliths are determined by growing temperatures and the $\delta^{18}O$ values of plant water according to the Shahack-Gross et al. (1996) equation:

$$t(^\circ C) = 5.8 - 2.8\,(\delta^{18}O_{\text{silica}} - \delta^{18}O_{\text{water}} - 40). \quad (2)$$

Stem phytoliths precipitate from water with $\delta^{18}O$ values unchanged from that of the soil water available to the plant over the entire growing season. However, leaf phytoliths are less ^{18}O-enriched than expected from their formation in contact with "modelled" leaf waters (Webb and Longstaffe, 2003), even though oxygen-isotope equilibration between silicic acid and water should be nearly instantaneous (Felipe et al., 2004). Instead, the $\delta^{18}O$ values of leaf silica appear to reflect equilibration with leaf water ($\delta^{18}O_{\text{leaf water for silica}}$) that is mixture of $\delta^{18}O_{\text{modelled leaf water}}$ and a proportion (X) of $\delta^{18}O_{\text{stem water}}$ from the veins:

$$\delta^{18}O_{\text{leaf water for silica}} = X(\delta^{18}O_{\text{stem water}}) + (1 - X) \times (\delta^{18}O_{\text{modelled leaf water}}). \quad (3)$$

This behaviour is not entirely unexpected as dissolved silica in plants is carried exclusively through the xylem and does not enter the phloem where nutrients and ^{18}O-enriched, partially evaporated waters are transported to the rest of the plant (Raven, 1983).

In the initial stages of cellulose formation, CO_2 enters the leaves through the stomatal pores and combines with ^{18}O-enriched leaf water ($\delta^{18}O_{\text{modelled leaf water}}$) to produce sucrose, which is translocated via the phloem to the intercalary meristems in the stem or at the base of developing leaves. Here, during the sucrose to cellulose transformation, a fraction of the oxygen atoms (p_{ex}) exchanges with the plant water at the site of this reaction. The $\delta^{18}O$ value of the water in these tissues depends on the proportion (p_x) of stem water to highly ^{18}O-enriched modeled leaf water. Stem water may be incorporated into cellulose directly during stem cellulose formation, or in the case of leaf cellulose, involved indirectly as bulk leaf waters comprise a mixture of modelled leaf water and stem (vein) waters.

A two-component mixing model can describe the amount of stem water versus leaf water incorporated into cellulose for any tissue (Barbour and Farquhar, 2000):

$$\delta^{18}O_{\text{cellulose}} = \delta^{18}O_{\text{stem water}} + (\delta^{18}O_{\text{modelled leaf water}} - \delta^{18}O_{\text{stem water}})(1 - (p_{ex})(p_x)) + \varepsilon_o. \quad (4)$$

Cumulatively, the average fractionation (ε_o) between cellulose and plant water present during each stage of formation is $+27 \pm 3‰$. The term $(p_{ex})(p_x)$ represents the percentage of stem water involved in cellulose synthesis. As it is very difficult to separate the values of p_{ex} and p_x in this equation, many researchers (e.g., Roden et al., 2000; Anderson et al., 2002) express this relationship as:

$$\delta^{18}O_{\text{cellulose}} = (f)(\delta^{18}O_{\text{stem water}}) + (1 - f) \times (\delta^{18}O_{\text{modelled leaf water}}) + \varepsilon_o, \quad (5)$$

where f represents the amount of stem water involved in cellulose synthesis. The f value also serves as a dampening factor, compensating for exchange with bulk-leaf water whose $\delta^{18}O$ values represent a mixture of stem and modelled leaf waters (Anderson et al., 2002). Anderson et al. (2002) demonstrated that the dampening factor (f) is inversely related to relative humidity. This behaviour occurs because under lower relative humidity conditions, $\delta^{18}O_{\text{modelled leaf water}}$ values are a poorer approximation of actual bulk-leaf water $\delta^{18}O$ values, which include a greater

proportion of stem water. In addition, under water-stressed conditions when plant growth rates are limited, sucrose may be transformed though a greater number of triose phosphate cycles before cellulose is formed, allowing for a greater amount of oxygen-isotope exchange (Barbour and Farquhar, 2000). Hence, under more humid conditions, as f approaches zero the $\delta^{18}O$ value of cellulose can be approximated by the $\delta^{18}O$ values of modelled leaf water plus ε_o:

$$\delta^{18}O_{cellulose} = (\delta^{18}O_{modelled\ leaf\ water}) + 27 \pm 3‰. \quad (6)$$

In this paper, we use the $\delta^{18}O$ values of coexisting cellulose and phytoliths in modern grasses to determine the $\delta^{18}O$ values of leaf and stem water independently from direct measurement. An ability to determine the oxygen-isotope compositions of leaf and stem waters using a combined cellulose-phytolith proxy would provide an additional means of estimating the $\delta^{18}O$ values of paleoprecipitation and more generally, past climatic conditions.

2. Methods

At the end of the 1995 and 1996 growing seasons, cellulose and silica phytoliths were extracted from the leaves and stems of *Calamovilfa longifolia*, a C_4 grass, which was collected across the North American prairies from a variety of climatic regimes (Fig. 1). *C. longifolia* typically grows in sandy, well-drained soils. The $\delta^{18}O$ values of cellulose from plants growing in this soil type are ideal for the reconstruction of summer climatic conditions, as the water feeding the plants should be biased towards summer precipitation events whose $\delta^{18}O$ values will correlate more strongly to temperature than soil waters comprising an annual mixture of precipitation events (McCarroll and Loader, 2004).

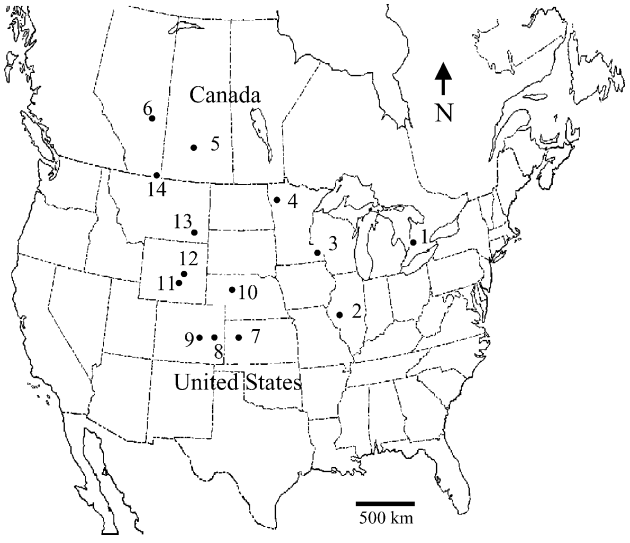

Fig. 1. Sample locations across North America. (1) Pinery Provincial Park, ON; (2) Longwoods Conservation Area, Havanna, IL; (3) Kellogg, MN; (4) Fertile, MN; (5) Dundurn, SA; (6) University of Alberta, Kinsella Ranch, AB; (7) Quinter, KS; (8) Aroya, CO; (9) Colorado Springs, CO; (10) Thedford, NE; (11) Rawlins, WY; (12) Kortes Dam, WY; (13) Broadus, MT; (14) Agriculture Canada Station, Onefour, AB.

Phytoliths were extracted from clean grasses via wet oxidation with sulfuric acid and hydrogen peroxide. Prior to analysis, the oxygen-isotope exchange procedure for opal-A described by Juillet-Leclerc and Labeyrie (1987) was employed to fix the oxygen isotopic composition of hydroxyl groups, which exchange with unstable Si–O bonds during dehydration. Subsequently, oxygen was liberated from the silica by reaction with BrF_5 at 600 °C (Clayton and Mayeda, 1963). A detailed account of these procedures and the calculations used to correct for oxygen-isotope exchange of the hydroxyl groups was provided by Webb and Longstaffe (2000, 2002). α-Cellulose extraction involved delipification with acetone, bleaching with sodium chlorite and acetic acid and hemi-cellulose removal with sodium hydroxide (Sternberg, 1989). Oxygen-isotope analyses of the cellulose was performed by on-line pyrolysis at 1300 °C using a Thermo Finnigan TC/EA and Delta^{plus} XL continuous flow mass spectrometer (Kornexl et al., 1999a). All results are reported in the standard delta notation:

$$\delta = [(R_{sample}/R_{standard}) - 1] \times 1000(‰), \quad (7)$$

where R represents $^{18}O/^{16}O$. Isotopic enrichment between two phases is expressed as:

$$\Delta^{18}O_{a-b} = \delta^{18}O_a - \delta^{18}O_b. \quad (8)$$

Reproducibility averaged ±0.2‰ for $\delta^{18}O_{phytolith}$ values and ±0.3‰ for $\delta^{18}O_{cellulose}$ values. Throughout the oxygen-isotope analysis of phytoliths, the $\delta^{18}O$ values of the laboratory quartz standard (NBS-28) maintained a consistent value of +9.7 ± 0.2‰. Cellulose $\delta^{18}O$ values were normalized against two laboratory standards: ANU sucrose $\delta^{18}O = +36.4‰$ (Farquhar et al., 1997) and Ennadai $\delta^{18}O = +22.2‰$ (Loader and Buhay, 1999). IAEA-C_3 cellulose was used as an indicator of accuracy. The reported $\delta^{18}O$ values of this material range from +31.3‰ to +32.8‰ (Buhay et al., 1995; Saurer et al., 1998; Kornexl et al., 1999b; Loader and Buhay, 1999; Sternberg et al., 2003), with an accepted value of +31.85‰ (Buhay et al., 1995). The average $\delta^{18}O$ value obtained in this study was +32.5 ± 0.5‰ ($n = 38$).

Calculations of $\delta^{18}O$ values for modelled leaf water from Eq. (1) were based on average temperature and relative humidity data for the entire growing season (May–August), as reported by Environment Canada (www.climate.weatheroffice.ec.gc.ca) and the on-line Climate Visualization System of the USA National Climatic Data Centre (http://lwf.ncdc.noaa.gov/oa/climate/climatedata.html). Recent experiments by Cappa et al. (2003) demonstrated that the value for kinetic fractionation between liquid and vapour water during molecular diffusion is very similar to that predicted by the kinetic theory of gases ($\alpha_{L-v} = 0.9691$ or $\alpha_{V-L} = 1.0319$). The resulting value of $\varepsilon_k = +32‰$ is greater than that presented by Merlivat (1978) ($\varepsilon_k = +28‰$), but is within the range of ε_k values

previously calculated by Webb and Longstaffe (2003) for the fractionation of plant water in *C. longifolia* based on the ε_k value determined by Merlivat (1978) and the calculations of Buhay et al. (1996) ($\varepsilon_k = +27‰$ to $+37‰$). The latter model incorporates variable leaf morphology and wind speed data, which were not available for the current study. The $\varepsilon_k = +32‰$ value has recently been used by several researchers to describe the fractionation associated with molecular diffusion from the stomata of a leaf (Cernusak et al., 2004; Pendall et al., 2005; Sheshshayee et al., 2005; Lai et al., 2006; Wright and Leavitt, 2006).

The $\delta^{18}O$ values of atmospheric water vapour were not measured for this study and are difficult to obtain for most areas. In addition, they can fluctuate widely in one region as a result of variations in temperature, air mass source, and absolute humidity (e.g., White and Gedzelman, 1984). For this study, average $\delta^{18}O$ values of atmospheric water vapour ($\delta^{18}O_{atm}$) over the growing season were calculated using the Majoube (1971) equation. Equilibrium with local soil water was assumed in this calculation, using $\delta^{18}O$ soil water values determined from Eq. (2), and measured temperature and $\delta^{18}O_{stem\ silica}$ values. Other researchers have demonstrated that for paleoclimate reconstruction, it is reasonable to assume that $\delta^{18}O_{atm}$ values are in equilibrium with soil water in continental regions (Edwards and Fritz, 1986; Anderson et al., 2002; Jahren and Sternberg, 2003).

3. Results and discussion

The $\delta^{18}O$ values for cellulose from 14 sites across North America are reported in Table 1. Table 1 also provides a summary of the $\delta^{18}O$ values for stem and leaf phytoliths previously reported by Webb and Longstaffe (2002) and available climate information for each study area.

3.1. The cellulose model

The initial stages of cellulose formation occur in the leaves. Both leaf- and stem-cellulose develop primarily in the presence of leaf water and hence do not differ greatly in their oxygen-isotope compositions at one location (Table 1). To assess the strength of the $\delta^{18}O_{modelled\ leaf\ water}$ signal preserved in the grass cellulose studied here, the $\delta^{18}O_{cellulose}$ values have been compared with those of modelled leaf water in Fig. 2. Included on this diagram are the lines that represent constant fractionation between leaf water and cellulose as described in Eq. (6) for varying values of ε_o ($+27 \pm 3‰$). Except for two locations, the data are bound by the upper and lower values of ε_o. Stem cellulose $\delta^{18}O$ values are slightly lower than leaf cellulose $\delta^{18}O$ values owing to their greater incorporation of stem waters during formation.

The data from Onefour Alberta, and Broadus Montana (Table 1) do not follow the general trend. Inaccuracies in the temperature and relative humidity data used in the calculation of $\delta^{18}O_{modelled\ leaf\ water}$ values are the most likely explanation for these two anomalous sites. Elevation differences and the effect of the rain-shadow from the Rocky Mountains can cause significant climatic variations between the remote sampling sites and the nearest weather stations in our database (Medicine Hat, Alberta for Onefour, Alberta and Billings, Montana for Broadus, Montana).

The values for f (stem water fraction) calculated from Eq. (5) and $\varepsilon_o = +27‰$, are listed in Table 1. For many of the grasses, the leaf cellulose appears to have formed entirely from leaf water having an oxygen-isotope composition of modelled leaf water. The average f values calculated for leaf cellulose for all sites (excluding Onefour, Alberta and Broadus, Montana) is -0.001 or -0.1%, suggesting that the water involved in leaf cellulose formation was on average slightly more enriched in ^{18}O than calculated. This outcome is not surprising. The measured $\delta^{18}O$ values of bulk-leaf water for grasses are commonly higher than modelled results because of progressive ^{18}O enrichment along the length of the leaf (Helliker and Ehleringer, 2000). It is important to note that for this study the $\delta^{18}O_{modelled\ leaf\ water}$ values were calculated using average growing season temperatures, and do not reflect daytime-high $\delta^{18}O$ values of leaf water reported in many studies. Our calculated f values are consequently much lower than typical amounts (0.22–0.46) reported by others (e.g., Roden et al., 2000; Anderson et al., 2002; Helliker and Ehleringer, 2002b; Sternberg et al., 2003). Although photophosphorylation, the initial process of photosynthesis, requires light, carbon fixation is a dark reaction, which can occur throughout the diurnal period (Geiger and Servaites, 1994). During the night, sucrose may be formed from the breakdown of starches, a process which has been demonstrated to be non-fractionating for oxygen isotopes (DeNiro and Cooper, 1989). In addition, it has been previously demonstrated that some C_4 monocots have low carbonic anhydrase activity, which restricts the isotopic equilibration between incoming CO_2 and ^{18}O-enriched leaf water (Gillon and Yakir, 2000). Partial equilibration would serve to lower the overall $\delta^{18}O$ values of the cellulose and create the appearance that it formed from leaf waters that are less ^{18}O enriched than expected under mid-day conditions. Hence, for this study, it is reasonable to use average daily temperature and relative humidity values in Eq. (1) when calculating the $\delta^{18}O$ values of leaf water involved in cellulose formation. Because silica phytoliths are deposited more or less continuously (Shahack-Gross et al., 1996), their $\delta^{18}O$ values reflect temperature and relative humidity conditions and plant water $\delta^{18}O$ values for the entire diurnal period. Hence, in order to compare plant water values involved in the synthesis of cellulose and phytoliths the average daily $\delta^{18}O$ values of modelled leaf water have been used.

Considering variations that may have arisen from the assumptions involved in obtaining the $\delta^{18}O_{atm}$ values used in the calculation of $\delta^{18}O_{modelled\ leaf\ water}$ values, it seems reasonable to presume that for these samples f is equal to zero and that leaf cellulose formed entirely from

Table 1
Measured and modelled temperature, relative humidity (RH) and $\delta^{18}O$ values (‰, VSMOW) of cellulose, phytoliths and plant water for *C. longifolia* from various sampling locations

Location	Year	Measured values[a]								Plant water values		Cellulose model			Leaf phytolith model		Values calculated entirely from the model				
		Temperature	Average RH		Surface	Cellulose		Phytoliths		Soil/stem	Modelled	*f*		Modelled	Leaf water	*X*		*X*	Leaf water		Soil
		M–A[b]	M–A[b]	J–A[b]	Water	Leaves	Stems	Leaves	Stems	Water	Leaf water	Cellulose		Leaf water	For silica	Leaf	RH	Leaf	For silica	Temperature	Water
		°C			$\delta^{18}O$	$\delta^{18}O$	$\delta^{18}O$	$\delta^{18}O$	$\delta^{18}O$	$\delta^{18}O$ Eq. (2)	$\delta^{18}O$ Eq. (1)	Leaf Eq. (5)	Stem Eq. (5)	$\delta^{18}O$ Eq. (6)	$\delta^{18}O$ Eq. (2)	Silica Eq. (3)	J–A[b] Eq. (10)	Silica Eq. (9)	$\delta^{18}O$ Eq. (12)	°C Eq. (2)	$\delta^{18}O$ Eq. (2)
Humid sites: min/max RH > 50/90%																					
1. Pinery, Ontario	1995	20	0.73	0.76	−7.2	30.6	29.3	32.4	28.1	−6.8	4.6	0.09	0.20	3.6	−2.5	0.59	0.72	0.63	−3.7	17	−8.0
Pinery, Ontario	1996	18	0.72	0.71	−7.2	29.5	28.0	28.7	26.4	−9.2	2.9	0.03	0.16	2.5	−6.9	0.80	0.82	0.79	−6.1	20	−8.4
Pinery, Ontario	1997	17	0.78	0.79	−7.2	29.4	29.1	31.7	27.5	−8.5	1.1	−0.14	−0.11	2.4	−4.3	0.62	0.73	0.64	−4.9	15	−9.1
2. Havannna, Illinois	1996	21	0.74	0.73	−6.2	32.4	31.0	32.3	27.3	−7.3	3.7	−0.15	−0.02	5.4	−2.3	0.60	0.69	0.58	−1.6	23	−6.6
3. Kellogg, Minnesota	1995	20	0.68	0.74	−8.0	32.4	31.0	29.7	27.2	−7.7	5.8	0.03	0.13	5.4	−5.2	0.81	0.81	0.77	−3.0	26	−5.5
4. Fertile, Minnesota	1995	18	0.68	0.71	−9.0	29.1	29.1	28.6	25.7	−9.9	3.6	0.11	0.11	2.1	−7.0	0.76	0.79	0.74	−6.0	21	−8.9
5. Dundum, Saskatchewan	1995	15	0.68	0.76	−11.5	28.6	29.5	29.3	23.9	−12.8	1.1	−0.04	−0.10	1.6	−7.4	0.63	0.68	0.55	−5.1	21	−10.5
6. Kinsella, Alberta	1995	14	0.69	0.75	−18.5	26.6	23.7	28.2	23.0	−14.1	−0.8	−0.03	0.19	−0.4	−8.9	0.62	0.69	0.57	−7.2	19	−12.4
7. Quinter, Kansas	1996	22	0.72	0.71	−5.9	31.2	30.8	29.3	26.3	−7.9	4.1	−0.01	0.02	4.2	−4.9	0.75	0.78	0.73	−3.8	25	−6.8
Arid sites: min/max RH < 50/90%																					
8. Aroya, Colorado	1996	21	0.64	0.66	−7.2	34.7	32.3	35.1	27.0	−7.6	7.6	−0.01	0.15	7.7	0.5	0.47	0.59	0.41	2.1	26	−6.0
9. Colorado Springs	1996	19	0.59	0.62	−5.8	35.0	34.6	33.2	27.5	−7.8	9.7	0.10	0.12	8.0	−2.1	0.64	0.67	0.54	1.4	29	−4.3
10. Thedford, Nebraska	1995	20	0.65	0.62	−10.5	32.7	31.2	33.3	26.1	−8.8	5.9	0.02	0.12	5.7	−1.6	0.50	0.62	0.45	−0.3	24	−7.5
11. Rawlins, Wyoming	1995	15	0.57	0.47	−15.6	32.0	32.3	36.9	23.6	−13.1	5.4	0.02	0.00	5.0	0.2	0.27	0.48	0.21	1.5	19	−11.8
12. Kortes Dam, Wyoming	1995	15	0.57	0.47	−16.4	31.4	28.8	38.9	22.5	−14.2	4.1	−0.02	0.12	4.4	2.2	0.12	0.43	0.12	2.1	15	−14.3
13. Broadus, Montanta	1995	20	0.60	0.55	−14.4	27.7	27.5	33.1	23.3	−11.6	5.2	0.27	0.28	0.7	−1.8	0.21	0.55	0.33	−4.2	13	−14.0
14. Onefour, Alberta	1995	16	0.57	0.56	−14.5	27.3	26.0	32.7	24.2	−12.2	6.1	0.32	0.39	0.3	−3.7	0.32	0.58	0.39	−5.1	12	−13.6

[a] Climate data, surface water $\delta^{18}O$ values, and phytolith $\delta^{18}O$ values were originally reported in Webb and Longstaffe (2002).

[b] Climate data is reported for the May to August (M–A) or July to August (J–A) growing periods.

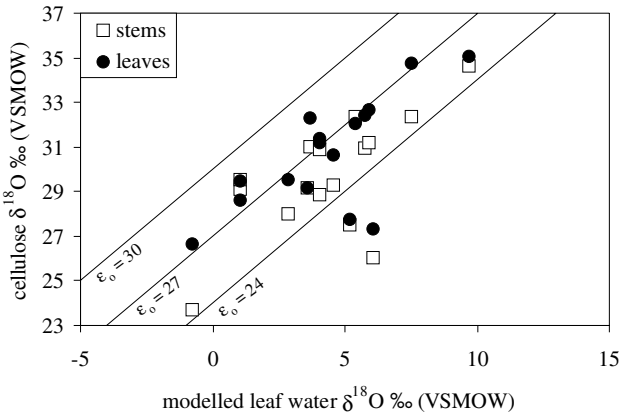

Fig. 2. Correlation between modelled leaf water $\delta^{18}O$ values calculated using Eq. (1) and measured leaf (●) and stem (□) cellulose $\delta^{18}O$ values. Error bars are smaller than the symbols. The solid lines represent the expected relationship for a constant fractionation between leaf water and cellulose as described by Eq. (6) for $\varepsilon_o = +27 \pm 3‰$.

$\delta^{18}O_{\text{modelled leaf waters}}$. However, calculated f values for stem cellulose are higher, averaging 0.08 or 8%, which suggests more immediate involvement of stem water in cellulose formation in these tissues (Table 1). This is not unexpected as the sucrose to cellulose transformation is associated with partial oxygen isotope exchange between the cellulose precursor molecules and water in the tissue of formation (Farquhar et al., 1998). Since it is highly improbable that stem and leaf cellulose from grasses can be separated from each other once they are deposited in sediments, it appears that for paleoclimate models, the most appropriate relationship to describe $\delta^{18}O_{\text{modelled leaf water}}$ values in terms of $\delta^{18}O_{\text{cellulose}}$ values for our data is Eq. (6) using $\varepsilon_o = +27‰$. This relationship can also be used to calculate modelled leaf water $\delta^{18}O$ values for Broadus, Montana and Onefour, Alberta.

3.2. The phytolith model

The ^{18}O enrichment of leaf silica relative to stem silica arises from the incorporation of ^{18}O-enriched leaf water into the leaf silica structure. The proportion of stem water involved in leaf silica synthesis (X) can be calculated with Eq. (3), using $\delta^{18}O_{\text{leaf cellulose}}$ values and Eq. (6) to calculate $\delta^{18}O_{\text{modelled leaf water}}$ values, and Eq. (2), using measured growing season temperatures (May–August) and $\delta^{18}O_{\text{stem silica}}$ or $\delta^{18}O_{\text{leaf silica}}$ values to calculate $\delta^{18}O_{\text{soil water}}$ and $\delta^{18}O_{\text{leaf water for silica}}$ values, respectively. Eq. (3) does not provide the proportion of stem water present in the leaf at any one time. Instead it is an approximation of the fit of $\delta^{18}O_{\text{modelled leaf water}}$ values to actual bulk leaf water $\delta^{18}O$ values over the growing season, assuming a mixture of two end-member $\delta^{18}O$ values for water in the leaf. In arid regions where elevated transpiration rates are sustained over the growing season, seasonal average leaf water $\delta^{18}O$ values are typically more enriched in ^{18}O relative to stem waters than in humid regions. This leads to an apparently smaller involvement of stem water in leaf silica synthesis. Hence, calculated values of X can be correlated to relative humidity (Fig. 3):

$$X = 1.7h - 0.6; \quad R^2 = 0.7, \tag{9}$$

where h is relative humidity expressed as a decimal fraction. Similar data for a C_3 grass species (*Ammophila breviligulata*) grown at the Ontario sampling site was available and appears to follow the same trend. However, it is possible that the relationship between X and relative humidity described in Eq. (9) will vary for species with significantly different venations and leaf water volumes.

Webb and Longstaffe (2002) previously demonstrated for the same data set that the ^{18}O enrichment of leaf phytoliths relative to stem phytoliths increases with the inverse of relative humidity:

$$\Delta^{18}O_{\text{leaf silica–stem silica}} = 12.5/h - 13.0; \quad R^2 = 0.9. \tag{10}$$

This allows for an independent estimate of relative humidity when measured values are unavailable. In both Eqs. (9) and (10), average late season (July–August) relative humidity values have been used rather than values for the entire growing season. Webb and Longstaffe (2002) demonstrated that late season relative humidity values produce a stronger correlation with leaf silica ^{18}O-enrichment. This may occur because the majority of phytolith silica forms late in the growing season (Shahack-Gross et al., 1996). It may also arise because late season average relative humidity more accurately reflects the variation in mid-day conditions between sites than the seasonal average relative humidity values. The latter values are buffered by cool, humid night conditions at the beginning and end of the growing season. By comparison and despite the possible increase in late season silica production, the $\delta^{18}O$ values of phytoliths were better correlated to the average temperatures over the entire growing season (May–August) than to the late summer period (Webb and

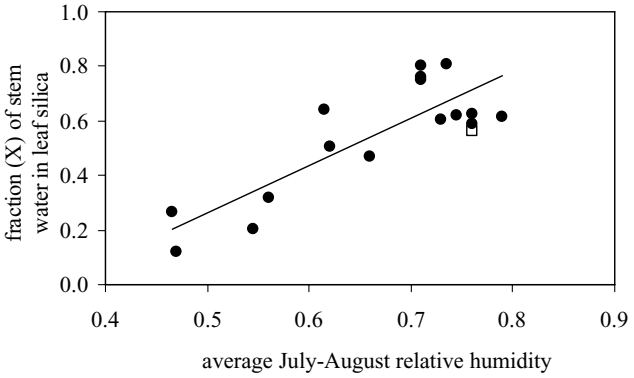

Fig. 3. Correlation between the proportion of stem water (X) involved in leaf silica formation versus average July to August relative humidity for *C. longifolia* (●) and *A. breviligulata* (□). The solid line is the regression through the data points for *C. longifolia*.

Longstaffe, 2002). These results may therefore be compared with the temperature of cellulose production throughout the entire growing season.

Since leaf and stem silica should form at nearly the same temperature, the spread in $\delta^{18}O$ values between silica and plant water should be equal for both stem and leaf silica. This relationship can be expressed as:

$$\delta^{18}O_{\text{stem water}} = \delta^{18}O_{\text{stem silica}} - \delta^{18}O_{\text{leaf silica}} + \delta^{18}O_{\text{leaf water for silica}}. \quad (11)$$

The relationships defined for the values of $\delta^{18}O_{\text{stem water}}$ in Eq. (11) and $\delta^{18}O_{\text{modelled leaf water}}$ in Eq. (6) can be substituted into Eq. (3) to form:

$$\delta^{18}O_{\text{leaf water for silica}} = (\delta^{18}O_{\text{leaf cellulose}} - 27) + (X(\delta^{18}O_{\text{stem silica}} - \delta^{18}O_{\text{leaf silica}})/(1 - X)). \quad (12)$$

X can be calculated from leaf silica and stem silica $\delta^{18}O$ values in combination with Eqs. (9) and (10). Eq. (12) can be used to calculate the $\delta^{18}O$ values of leaf water in which leaf silica formed. These values can then be entered into the Shahack-Gross et al. (1996) phytolith-plant water paleothermometer (Eq. 2) to obtain growing season temperatures.

The calculated and measured temperatures are compared for each of the sampling sites in Fig. 4:

$$t_{\text{modelled}} = 1.0(t_{\text{measured}}) + 3.6; \quad R^2 = 0.4, \quad (13)$$

where t is the temperature in degrees Celsius. The correlation is poor ($R^2 = 0.4$) but the data generally cluster about a 1:1 relationship. The range of modelled temperatures extends to 29 °C, which is 7 °C higher than any observed seasonal average temperature and 10 °C higher than the measured temperature at that site (Colorado Springs, CO). However, at the majority of sites (12 out of 16) the modelled temperatures are within 5 °C of the measured temperature.

It is likely that the measured temperature for each site does not reflect the growing temperature sufficiently well. In some instances, the temperature data were obtained ~100 km distant from the sampling site. In addition, the growth of grasses is partly regulated by temperature (Sutcliffe, 1977). Growing conditions may not have been favourable during some periods of the May–August season. It is possible that the calculated temperatures more closely reflect those of optimal plant growth, rather than an average for the growing season. It has been demonstrated that C_4 grasses germinate more readily at temperatures of 27 °C, with maximum rates of photosynthesis and dry-matter accumulation generally occurring between 30 and 35 °C (Black, 1971; Harper, 1977; McWilliam, 1978). Furthermore, *C. longifolia* often grows in bare sandy soils, which have a poor thermal conductivity. For the Pinery Provincial Park site, sand-surface temperatures during the midsummer months could be up to 20 °C higher than the air temperature 30 cm above the surface (Webb and Longstaffe, 2003).

If the modelled temperatures are used in combination with $\delta^{18}O$ values of stem silica and Eq. (2) we are able to calculate the $\delta^{18}O$ values of soil water utilized by the grasses. Fig. 5 illustrates the modelled soil water $\delta^{18}O$ values plotted versus measured surface water $\delta^{18}O$ values. The latter data should provide a reasonable approximation for the average $\delta^{18}O$ values of precipitation during the growing season. In contrast to the calculated temperatures (Fig. 4), there is a better correlation between modelled and measured surface water $\delta^{18}O$ values, although the slope is less than one:

$$\delta^{18}O_{\text{modelled soil water}} = 0.7(\delta^{18}O_{\text{surface water}}) - 2.4; \quad R^2 = 0.8. \quad (14)$$

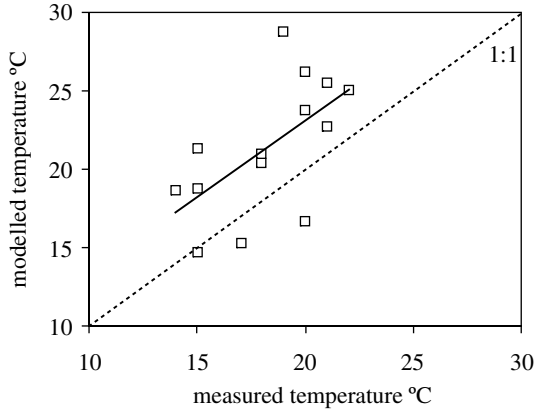

Fig. 4. Calculated versus measured average growing season temperatures (May–August) (see text). Results for the Broadus, Montana and Onefour, Alberta sites have been omitted (see text). The dotted line represents the 1:1 relationship that would be expected if the temperatures calculated from the model accurately reflected the measured growing season temperatures.

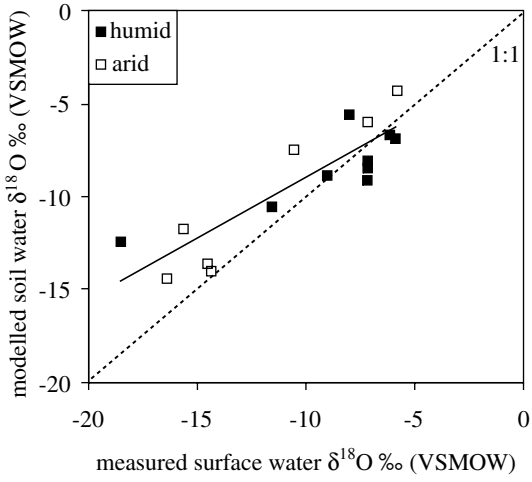

Fig. 5. Calculated modelled soil water $\delta^{18}O$ values (see text) versus measured surface water $\delta^{18}O$ values for humid (■) and arid (□) sites. The solid line (Eq. 14) is the regression though the data for all sites and the dotted line represents the anticipated 1:1 relationship.

This discrepancy is related to differences in the $\delta^{18}O$ values of surface water versus soil water utilized by the plant. As illustrated by Fig. 5, modelled soil water $\delta^{18}O$ values for arid sites plot above the 1:1 line, consistent with greater evaporative ^{18}O-enrichment of soil water relative to surface water.

Hence, it is possible in the absence of climate data to predict the $\delta^{18}O$ values of water utilized by a plant using only the $\delta^{18}O$ values of phytoliths and cellulose. There may be errors in our initial calculation of $\delta^{18}O_{\text{modelled leaf water}}$ introduced by assumptions of $\delta^{18}O_{atm}$ and average growing season temperatures. However, since both silica and cellulose formed within the same plant, the model presented here eliminates the need to know absolute $\delta^{18}O_{\text{modelled leaf water}}$ values.

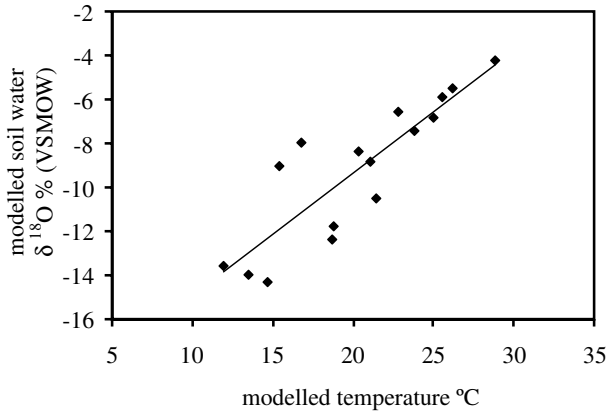

Fig. 6. The relationship between modelled temperature and $\delta^{18}O$ soil water values, as calculated using the cellulose-phytolith $\delta^{18}O$ model (see text).

3.3. Implication for paleoclimate studies

Although Helliker and Ehleringer (2002a,b) have studied the effects of climate on the $\delta^{18}O$ values of cellulose in modern grass species, the majority of paleoclimate reconstruction efforts that use the $\delta^{18}O$ values of cellulose have been based on woody tree-ring cellulose (e.g., Burk and Stuiver, 1981; Anderson et al., 2002; Waterhouse et al., 2002; McCarroll and Loader, 2004). Unfortunately, trees and other dicotyledons are in general, poor phytolith producers and hence the model presented here is ill-suited for tree-ring studies. By comparison, grasses and sedges are abundant producers of both phytoliths and cellulose, which accumulate in underlying soils and peat deposits. Accordingly, the changes in $\delta^{18}O$ values of these plant residues with increasing depth within a soil or peat profile can be used in this model to infer climate changes over time in grassland environments or peat deposits.

The wide distribution of grasslands in mid-continental North America provides a special opportunity to examine spatial climate gradients across these terrains. The shallow rooting systems of grasses (approximately 1 m for the majority of roots for *C. longifolia*) tend to feed on precipitation events (Golluscio et al., 1998). In addition, grasses, such as *C. longifolia*, which thrive on well-drained sandy soils, will have more access to soil water with $\delta^{18}O$ values similar to summer precipitation (Webb and Longstaffe, 2003). The $\delta^{18}O$ values of summer precipitation are more easily correlated to temperature than well-mixed soil waters that may be tapped by deeper-rooted trees or retained at shallower levels in clay rich soils. The modelled soil water $\delta^{18}O$ values obtained in this study are plotted versus the modelled temperature values (Fig. 6):

$$\delta^{18}O_{\text{modelled soil water}} = 0.56\ t_{\text{modelled}} - 20.6; \quad R^2 = 0.8, \quad (15)$$

where t is the temperature in degrees Celsius. The resulting relationship is very similar to equations that relate the $\delta^{18}O$ values of precipitation to surface temperatures (Rosanski et al., 1993, 0.58‰/°C; Fricke and O'Neil, 1999, 0.55‰/°C or 0.42‰/°C for summer and 0.58‰/°C for winter; Welker, 2000, 0.46–0.55‰/°C).

Physical separation of phytoliths that originated from these two plant tissues is required to obtain $\delta^{18}O$ values of stem and leaf phytoliths from soils. Many stem and leaf phytoliths can be distinguished based on their three-dimensional physical characteristics, but other phytolith morphologies are not mutually exclusive to one tissue or another (Brown, 1984). Further study is warranted to determine whether the above relationships can be applied to handpicked suites of phytoliths with specific morphologies that may not include 100% of leaf or stem phytoliths.

There is also potential for variation in the $\delta^{18}O$ values of water in different cells within a leaf. These variations may be preserved in the $\delta^{18}O$ values of phytoliths whose distinct morphologies are determined by the shape of the cell. For example, leaf water adjacent to the stomata is assumed to be among the most ^{18}O-enriched; hence a bias in the $\delta^{18}O$ values of leaf silica could be created if only phytoliths originating from stomata guard cells are selected from a soil phytolith assemblage.

Application of this model to paleoclimate studies is further complicated by the impossibility of segregating leaf from stem cellulose in a soil deposit. However, it appears that stem and leaf cellulose from grasses have very similar $\delta^{18}O$ values, which are dominated by leaf water $\delta^{18}O$ values (this study; Shahack-Gross et al., 1996). The similarities between $\delta^{18}O$ values of stem and leaf cellulose persist despite the fact that leaves and stems represent the two extremes for $\delta^{18}O$ values of water within the transpiring and non-transpiring tissues of *C. longifolia* (Webb and Longstaffe, 2003). Although using the average $\delta^{18}O$ values of leaf and stem cellulose would not significantly change the relationship between cellulose and leaf water described by Eq. (6), approximately 80% of the biomass of *C. longifolia* occurs in non-transpiring tissues, the majority of which occur underground as roots and rhizomes (Maun, 1985; Webb and Longstaffe, 2002). Hence, for mixtures of cellulose preserved in sediments, ε_o may appear to be less than +27‰. Further study of the amount of cellulose formed in each of the plant tissues and the contribution of cellulose

from those tissues to the overall $\delta^{18}O_{cellulose}$ value of the whole plant is needed.

4. Conclusions

The oxygen-isotope compositions of leaf cellulose and silica record a complex signal of the $\delta^{18}O$ value of the soil water that feeds the plants and relative humidity conditions affecting the ^{18}O-enrichment of leaf waters. A comparison of the $\delta^{18}O$ values of these two plant products has enabled us to deduce the proportion of leaf- versus stem-water $\delta^{18}O$ values preserved in leaf silica under varying relative humidity conditions. Using the $\delta^{18}O$ values of leaf cellulose as a proxy for the $\delta^{18}O$ value of leaf water permits the calculation of growing temperatures, which can then be used in conjunction with stem silica $\delta^{18}O$ values to calculate soil water $\delta^{18}O$ values. This procedure circumvents some of the uncertainties in interpreting climate based on the $\delta^{18}O$ values of plant cellulose. In particular, errors introduced by fluctuations in the $\delta^{18}O$ values of precipitation arising from diurnal/seasonal variations and mixing in the soil water reservoir are minimized. The model proposed here holds promise for obtaining quantitative temperature, relative humidity and soil water $\delta^{18}O$ values from ancient environments through the oxygen-isotope analysis of silica phytoliths and cellulose preserved in soils.

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