Carbon and oxygen isotope ratios of tree ring cellulose along a precipitation transect in Oregon, United States

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[1] The carbon and oxygen isotopic compositions of tree ring cellulose were examined for trees along a precipitation gradient in western Oregon, United States. Two years of cellulose from four sites dominated by coniferous forests ranging in precipitation from 227 to 2129 mm were sampled in conjunction with studies that measured the $\delta^{18}O$ and $\delta^{13}C$ of ecosystem respiration. The mean tree ring cellulose $\delta^{13}C$ varied from -22.1 to -26.3‰ among sites and showed enrichment with decreasing water availability across the transect. The $\delta^{13}C$ in cellulose varied across the precipitation transect in a similar pattern to the $\delta^{13}C$ of leaf and root tissues as well as ecosystem respiration, although tree ring cellulose was enriched in ^{13}C by over 3‰ compared to other organic matter components. The mean tree ring cellulose $\delta^{18}O$ varied from 28.1 to 30.3‰. However, trends of cellulose $\delta^{18}O$ change with water availability were obscured by differences in stem water $\delta^{18}O$. When calculated as deviation from stem water ($\delta^{18}O_{cellulose} - \delta^{18}O_{stem\ water}$) the differences in evaporative enrichment between sites was more pronounced (range of 9.6‰). The limited observed variation in tree ring cellulose $\delta^{18}O$ of field grown trees despite large site difference in stem and leaf water $\delta^{18}O$ across the transect agreed with predictions from a mechanistic model. Tree ring records of cellulose $\delta^{18}O$ may provide useful proxy information regarding humidity and site water balance especially if combined with $\delta^{13}C$ records that also vary with plant water status.

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1. Introduction

[2] The isotopic compositions of tree ring cellulose are widely used to study climate variability, since individual tree rings can be accurately dated (with sub-annual resolution) and cellulose once deposited is immobile. The three isotopic tracers in cellulose (carbon, hydrogen, and oxygen) have been correlated with climatic drivers, often leading to robust conclusions that allow climatic reconstruction in a region [*Anderson et al.*, 1998; *Leavitt and Long*, 1991; *McCarroll and Pawellek*, 2001; *Robertson et al.*, 2001; *Saurer*, 2003], but occasionally studies have been at odds with each other especially with regard to whether or not a specific climatic parameter is recorded in cellulose. For example, some studies have concluded that cellulose isotopes recorded interannual variations in atmospheric humidity [*Edwards and Fritz*, 1986], whereas other studies have concluded that cellulose recorded water source without influence of humidity [*DeNiro and Cooper*, 1989]. *Roden et al.* [2000] provided a model that quantitatively explained the extent to which environmental drivers influenced the hydrogen and oxygen isotopic composition of cellulose.

[3] The carbon isotopic composition of plant organic matter is influenced by a number of environmental factors [*Farquhar et al.*, 1989; *Ehleringer et al.*, 1993]. The average $\delta^{13}C$ value in organic matter is influenced primarily by $\delta^{13}C$ of atmospheric CO_2, diffusion of CO_2 through stomata, and enzymatic discrimination during the irreversible step of CO_2 fixation. Studies have shown that ^{13}C discrimination in C_3 plants varies with water stress [*Ehleringer and Cooper*, 1988; *Saurer et al.*, 1995], solar radiation [*Ehleringer et al.*, 1986], and plant nutrition [*Livingston et al.*, 1999]. Thus $\delta^{13}C$ values of plant organic matter have been correlated with rainfall amount [*Stewart et al.*, 1995], vapor pressure deficit [*Comstock and Ehleringer*, 1992], canopy position [*Buchmann et al.*, 2002; *Fessenden and Ehleringer*, 2003] hydraulic conductivity associated with tree height [*Yoder et al.*, 1994] and water use efficiency [*Hubick et al.*, 1986; *Ehleringer et al.*, 1990; *Feng*, 1999].

[4] The oxygen isotopic composition of organic matter is also influenced by a number of environmental factors,

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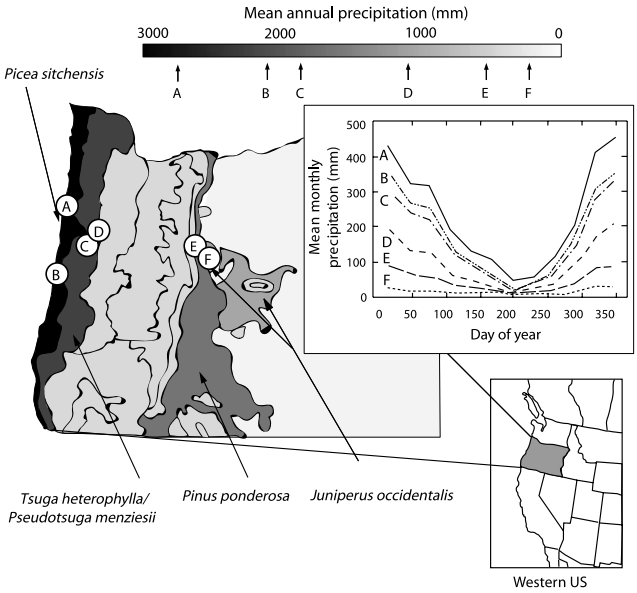

Figure 1. Location of study sites in Oregon. Figure is from *Bowling et al.* [2002] (used by permission, *Oecologia*, *131*, 113–124, DOI 10.1007/s00442-001-0851-y, Figure 1). All six sites represent the full OTTER network. Sites A and D were not part of the current study. Adapted from *Franklin and Dyrness* [1988].

including the $\delta^{18}O$ in meteoric water, the $\delta^{18}O$ of atmospheric vapor, atmospheric humidity and vapor pressure deficit [*Farquhar and Lloyd*, 1993; *Roden et al.*, 2000]. The $\delta^{18}O$ values in plant organic matter have been correlated with mean annual temperature [*Burk and Stuiver*, 1981], relative humidity [*Edwards and Fritz*, 1986; *Robertson et al.*, 2001; *Lipp et al.*, 1996], transpiration rates [*Barbour et al.*, 2000] and water balance [*White et al.*, 1994], presumably all through contributions of source water (the xylem water in suberized stems) and leaf water enrichment.

[5] While it is evident that both $\delta^{13}C$ and $\delta^{18}O$ in plant organic matter can be used as interrelated climate proxies, it is not evident how these parameters will vary along the complex precipitation gradients [*MacFarlane et al.*, 2004] that typify the coastal-to-interior gradients found on all continents. This is because oxygen isotope patterns in tree rings need not exhibit a unidirectional pattern if both water source values and humidity exhibit contrasting patterns along the transect. Similarly, carbon isotope values need not exhibit a monotonic relationship with precipitation if offsets in phenology and seasonality of primary growth periods are restricted in their duration at some sites and extended throughout the entire year at other sites.

[6] This project was conducted along one such climatic gradient, the North American Oregon Transect Ecosystem Research (OTTER [*Peterson and Waring*, 1994]) from coastal to central Oregon, USA. Along this coastal-to-interior transect, there is a substantial moisture gradient (differences of ~2500 mm in mean annual precipitation over 250 km). Previous studies along the Oregon transect have established clear patterns relating site water balance to primary productivity [*Running*, 1994; *Runyon et al.*, 1994; *Peterson and Waring*, 1994; *Anthoni et al.*, 1999] and to the stable isotopic composition of ecosystem respiration [*Bowling et al.*, 2002, 2003a; *McDowell et al.*, 2004b]. Each site along this transect was dominated by a single species of coniferous tree, but the dominant species of the forest differed across sites (Figure 1).

[7] Our first objective was to test the hypothesis that both $\delta^{13}C$ and $\delta^{18}O$ in tree ring cellulose increase with aridity along the precipitation transect owing to the effects of reduced soil moisture and humidity at the dry sites on both reducing stomatal conductance and the ratio of leaf-internal to atmospheric CO_2 (c_i/c_a effects on $\delta^{13}C$ [*Farquhar et al.*, 1989]) and in enhancing evaporative enrichment in $\delta^{18}O$ of leaf and soil waters. The Oregon transect provides an ideal opportunity to evaluate how much the $\delta^{18}O$ in source water and humidity alter the $\delta^{18}O$ in tree ring cellulose because of large differences in meteoric water $\delta^{18}O$, humidity and precipitation (Figure 2). The coastal sites have a milder climate than the inland sites, especially during winter months when the majority of precipitation falls. Site differences in precipitation and humidity suggest that evaporative enrichment should differentially modify leaf water $\delta^{18}O$ across the transect (Figure 2).

[8] Our second objective was to determine whether variation in tree ring cellulose $\delta^{18}O$ across the transect can be predicted using an existing model developed by *Roden et al.* [2000]. Environmental and isotopic information collected across the Oregon transect was used to field test this mechanistic model that describes the factors affecting the $\delta^{18}O$ in organic matter. This model has been tested previously under controlled environmental conditions [*Roden and Ehleringer*, 1999b; *Helliker and Ehleringer*, 2002] and field conditions using angiosperms [*Roden and Ehleringer*, 2000]. This study provides an opportunity to test this model under field conditions using gymnosperms, which are widely used in tree ring studies. Data collected across this transect from other studies [*Bowling et al.*, 2002, 2003a, 2003b; *McDowell et al.*, 2004a, 2004b] were used to test model predictions of tree ring cellulose $\delta^{18}O$ values.

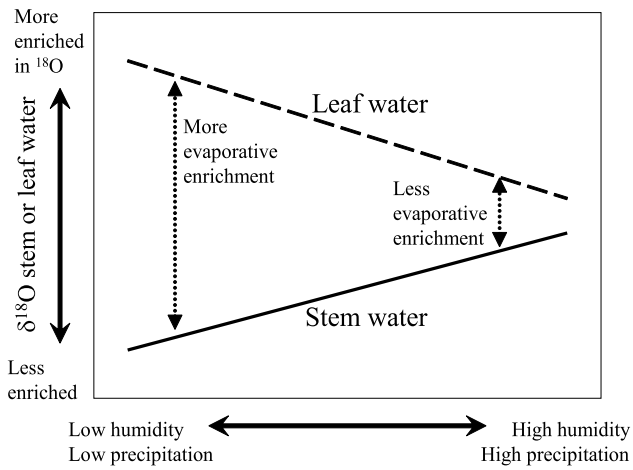

Figure 2. Potential effects of environmental factors across a precipitation transect (like OTTER) on stem and leaf water $\delta^{18}O$. In this diagram, precipitation is more depleted in ^{18}O at the inland sites owing to altitude and continental effects on meteoric waters [see *Bowling et al.*, 2003a].

Table 1. Details for Sites Utilized[a]

Site Descriptor	Dominant Species	Location and Elevation	PRISM Modeled 30-Year Mean Precipitation, mm	Approximate Age of Trees, years	Site Code From *Bowling et al.* [2002]
Spruce	*Picea sitchensis Tsuga heterophylla*	44°07′N, 124°07′W, 300 m	2129	20–22	B
Douglas-fir	*Pseudotsuga menziesii*	44°35′N, 123°35′W, 290 m	1892	12	C
Pine	*Pinus ponderosa*	44°30′N, 121°37′W, 941 m	523	70–300	E
Juniper	*Juniperus occidentalis*	44°18′N, 121°20′W, 930 m	227	30–100	F

[a]Sites correspond to those of *Bowling et al.* [2002] and differ from sites on the original Oregon transect. The pine site is an Ameriflux long-term CO_2 flux study site (Metolius Research Natural Area).

[9] Previous studies [*Bowling et al.*, 2002, 2003a, 2003b; *McDowell et al.*, 2004a, 2004b] utilized these same field sites to study the effects of site water balance and vapor pressure deficits on both the carbon and oxygen isotopic composition of ecosystem respiration ($\delta^{13}C_R$ and $\delta^{18}O_R$ respectively). They found that the $\delta^{13}C$ of carbon stocks (leaves, fine roots, soil organic matter etc.) as well as $\delta^{13}C_R$ was correlated with precipitation and vapor pressure deficit through stomatal regulation of gas exchange [*McDowell et al.*, 2004b]. In addition, the site differences in vapor pressure deficits affected $\delta^{18}O_R$ through evaporative enrichment that modified the primary effects of meteoric water $\delta^{18}O$ inputs [*Bowling et al.*, 2003a, 2003b]. This study was performed during the same study period and on the same sites as that of *Bowling et al.* [2002, 2003a, 2003b] and *McDowell et al.* [2004a, 2004b] in order to link the tree ring results with existing data on the carbon and oxygen isotopic composition of ecosystem respiration and organic matter, as well as to use their measurements of microclimate and $\delta^{18}O$ of ecosystem waters to parameterize the *Roden et al.* [2000] cellulose model.

2. Methods

2.1. Site Descriptions

[10] The coastal ''spruce site'' was dominated by a relatively young stand of even-aged *Picea sitchensis* (Table 1). The ''Douglas-fir site'' in the Tum Tum tree farm located in the central Oregon Coast Range was also a young stand of even-aged *Pseudotsuga menziesii*. The ''pine site'' in the Metolius Research Natural Area on the eastern side of the Cascade mountain crest was dominated by *Pinus ponderosa* of varying ages. The pine site is an AmeriFlux site, where eddy fluxes and soil and plant process rates were also measured and used to interpret the *Bowling et al.* [2002, 2003a, 2003b] and *McDowell et al.* [2004a, 2004b] studies. The ''juniper site'' in central Oregon was dominated by stands of *Juniperus occidentalis* of varying ages (Table 1). These four sites were a subset of the six used by *Bowling et al.* [2002, 2003a, Figure 1] and *McDowell* [2004a, 2004b].

2.2. Sample Processing

[11] In November of 2001, six healthy-looking trees were selected in the proximity of the air sampling towers at each site [see *Bowling et al.*, 2002]. Four cores (12 mm diameter) were obtained from the four cardinal directions at breast height of each tree. Owing to small growth rings for juniper trees, six cores were obtained for each tree at this site to provide sufficient material for analysis. Since the spruce and Douglas-fir sites were plantations, the variability in ages and growth rates between trees at these sites was minimal. The ages in the pine and juniper sites varied substantially more, and trees were selected if they were either a canopy dominant or subdominant, but not if they were very large (and old). Very old trees tend to have narrow tree rings [*Fritts*, 1976] making it difficult to obtain enough material when subdividing the annual growth (i.e., latewood and earlywood).

[12] The last two years of growth (2000, 2001) were subdivided into earlywood and latewood on the basis of position and visual assessments of wood density (under a 20x dissecting microscope). The latewood portion of the ring was distinctly darker than earlywood in these conifers. A portion of the ring between the subdivisions was discarded (>1/3 of the ring) to ensure distinctiveness of sub-sampling. The amount of sample obtained from each tree ring varied with width. The spruce and Douglas-fir sites had trees with particularly large growth increments (>10 mm) and some juniper rings were less than 0.5 mm in width. The samples from the four cardinal directions on a single tree were pooled and cut into small pieces with a razor blade. The samples were dried at 70°C for 48 hours and ground to a fine powder with a ball mill (Wig-L-Bug, Crescent, Elgin, Illinois).

[13] Approximately 50–100 mg (if available, some juniper samples were as small as 15 mg) of ground sample was loaded into fiber filter bags (ANKOM, Fairport, New York) and heat-sealed for cellulose extraction as described in detail by *Leavitt and Danzer* [1993]. Briefly, the filter bags were placed in a Soxhlet apparatus to reflux a 2:1 solution of toluene:ethanol for a 24-hour period followed by a period of drying and another 24-hour period of extraction (for lipids and resins) with 95% ethanol. The bags were dried and then boiled in water for 1 hour to extract soluble sugars and low molecular weight polysaccharides. The samples were then ''bleached'' using a strong sodium chlorite/acetic acid solution that was periodically replaced over a 4-day extraction (to extract lignin and other N containing compounds). Following these treatments the extracted material was in the form of holocellulose. To obtain pure α-cellulose the sample was exposed to a strong (17% w/v) NaOH solution followed by an acetic acid solution to neutralize the pH with each step followed by extensive rinsing with double distilled water. The α-cellulose was dried at 70°C for 48 hours.

2.3. Analysis of Stable Carbon and Oxygen Isotope Ratios

[14] For $\delta^{18}O$ measurements, 90–110 μg of α-cellulose was loaded into silver capsules and converted to CO by pyrolysis [*Saurer et al.*, 1998] in a hot (1400°C) alumina/

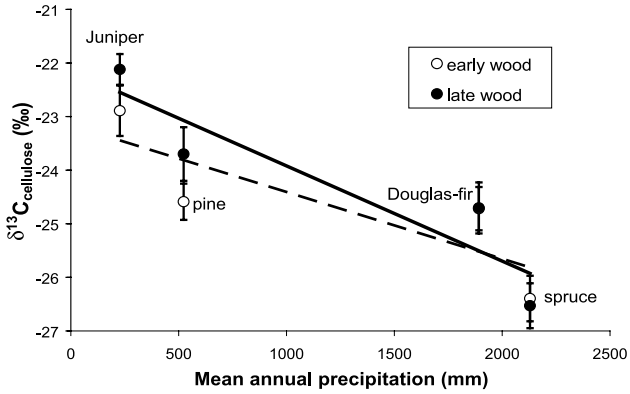

Figure 3. Mean carbon isotopic composition of tree ring cellulose across the OTTER transect (± standard deviation, n = 12). Values for 2000 and 2001 are pooled since there was no significant difference ($p > 0.05$) between years for either latewood or earlywood. Correlations between cellulose $\delta^{13}C$ and precipitation were significant ($p < 0.01$) for both latewood (equation: $\delta^{13}C_{cell} = -0.0018 \times$ mean precipitation -22.14) and earlywood (equation: $\delta^{13}C_{cell} = -0.0012 \times$ mean precipitation -23.16).

glassy carbon reactor (Thermo-Finnigan TC/EA) and separated from other gases in a 0.6-m molecular sieve 5A gas chromatography (GC) column connected to a Finnigan MAT deltaPlus XL isotope ratio mass spectrometer. Mass spectrometry was performed at the Stable Isotope Ratio Facility for Environmental Research at the University of Utah. For $\delta^{13}C$ measurements, approximately 1 mg of α-cellulose was loaded into tin capsules and combusted on an elemental analyzer (Carlo-Erba 1110, Milan) coupled to a Finnigan MAT delta Plus isotope ratio mass spectrometer. The precision of α-cellulose standards run with the samples for $\delta^{18}O$ was 0.23‰ (standard deviation, n = 36) and for $\delta^{13}C$ was 0.16‰ (n = 13).

2.4. Leaf, Stem, and Atmospheric Water Sampling

[15] To obtain source water values from trees at each site, stems between 5 and 10 mm in diameter and 5- to 7-cm long [see *Bowling et al.*, 2003a], were collected and stored in screw top vials covered in wax film, and refrigerated or frozen until the water could be extracted by cryogenic distillation under vacuum [*Ehleringer et al.*, 2000]. At each site, stems were sampled approximately 4 times during the growing season (April to September) in both 2000 and 2001. During August 2001, sun and shade needles were sampled in the morning and mid-day over a 2-day period at each site. Needles were stored, frozen and extracted for water in the same manner as stems. Water vapor was collected cryogenically at each site during the August 2001 field campaign using the method of *Helliker et al.* [2002]. All water samples were analyzed for $\delta^{18}O$ as described by *Fessenden et al.* [2002].

2.5. Micrometeorological Measurements

[16] At each site during 2001, measurements of air temperature and relative humidity (HMP45A, Vaisala, Inc. Woburn, Massachusetts) at the top of the canopy were measured every 5 s and stored as hourly averages with a data logger (CR23X, Campbell Scientific, Inc. Logan, Utah).

2.6. Model Description

[17] The water samples and meteorological information described above were collected to parameterize a model that predicts $\delta^{18}O$ of cellulose [*Roden et al.*, 2000] on the basis of predictions of the $\delta^{18}O$ at the evaporating surface of the leaf [*Craig and Gordon*, 1965; *Flanagan et al.*, 1991; *Roden and Ehleringer*, 1999a]. Predicting cellulose $\delta^{18}O$ utilizes the fractionation factor for the incorporation of ^{18}O into organic matter (27‰ [from *Sternberg*, 1989; *Yakir and DeNiro*, 1990] and the proportional exchange with water at the site of cellulose synthesis (stem water for tree rings, $f_o = 0.42$ [from *Roden and Ehleringer*, 1999b] [see also *Yakir and DeNiro*, 1990; *Helliker and Ehleringer*, 2002]).

[18] In our analysis we used both the mean environmental values (split between early season (April–June) and late season (July–September)) and the range of possible modeled outcomes of cellulose $\delta^{18}O$ (split between earlywood and latewood) based on the range of measured environmental values. Only daytime values of temperature and humidity were used in the model since that was considered the period of active photosynthesis. Although model predictions have no variance, the input parameters can vary widely and so we used the range of possible predictions as a way to provide some estimate of model output sensitivity.

3. Results

3.1. Carbon Isotopes

[19] The $\delta^{13}C$ values of tree ring cellulose were more negative (depleted in ^{13}C) at sites with consistently greater precipitation (Figure 3). There were no significant differences ($p > 0.05$) between samples collected in 2000 and 2001 and so the data for both years were pooled in Figure 3 (and subsequent figures). The similarity in $\delta^{13}C$ for these 2 years was reasonable since environmental conditions (air temperature and precipitation) were similar for both 2000 and 2001 at locations near each site (data (not shown) from nearby weather stations). Differences between sites were highly significant ($p < 0.05$, ANOVA using a Tukey multiple comparison test, SPSS Inc.) with the exception of the earlywood $\delta^{13}C$ values for the pine and Douglas-fir sites. Data for earlywood $\delta^{13}C$ were significantly different (more depleted in ^{13}C) than latewood at the pine and juniper sites only. The coastal spruce site and the inland juniper sites differed in latewood cellulose $\delta^{13}C$ by over 4.4‰.

[20] The $\delta^{13}C$ of fine root organic matter, tree ring cellulose and ecosystem respiration were correlated with $\delta^{13}C$ of sun needles (Figure 4, organic matter and respiration data from *Bowling et al.* [2002]). Latewood cellulose was consistently more enriched in ^{13}C than other components of tree organic matter or respiration (Figure 4). All components of tree organic matter $\delta^{13}C$ followed the same general trends with site water status, with more enriched $\delta^{13}C$ observed at the drier sites.

3.2. Oxygen Isotopes

[21] There were no significant differences ($p > 0.05$) in cellulose $\delta^{18}O$ between samples collected in 2000 and 2001

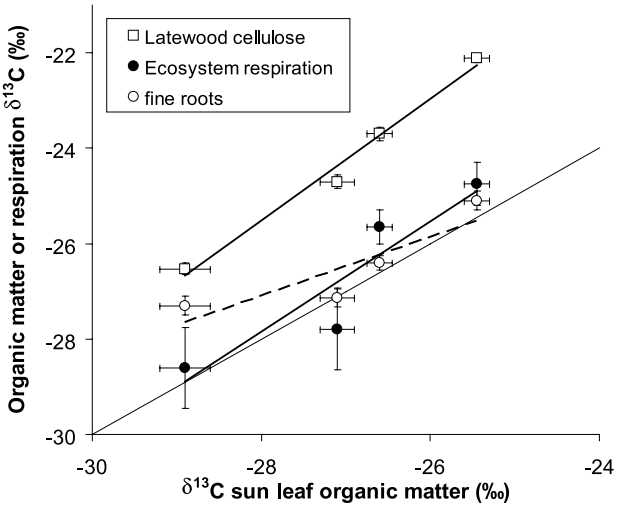

Figure 4. Mean carbon isotopic composition of tree ring cellulose, fine root organic matter and ecosystem respiration as a function of sun leaf organic matter $\delta^{13}C$ for each site (± standard error, n = 12 to 15 for organic matter). Ecosystem respiration, fine root, and sun leaf $\delta^{13}C$ data are from *Bowling et al.* [2002]. The dashed line represents the 1:1 line. Regression analysis (SPSS) produced a slope of 1.28, 1.16, and 0.613, a Pearson's correlation coefficient (SPSS) of 0.99 ($p = 0.007$), 0.924 ($p = 0.076$), and 0.880 ($p = 0.120$) for the relationships between $\delta^{13}C$ of sun leaf organic matter and $\delta^{13}C$ of latewood cellulose, $\delta^{13}C$ of ecosystem respiration, and $\delta^{13}C$ of fine root organic matter respectively.

and so the data for both years were pooled in Figure 5. Tree ring cellulose $\delta^{18}O$ differed by as much as 2‰ between sites. Differences in latewood $\delta^{18}O$ between sites were significantly different ($p < 0.05$) with the exception of the spruce and juniper site comparisons. Very few site differences were significant for earlywood $\delta^{18}O$ except when compared to the pine site. Although earlywood tended to be more enriched in $\delta^{18}O$ than latewood the differences were rarely significant. There were no clear trends in tree ring cellulose $\delta^{18}O$ with differences in site water availability.

[22] The lack of trends between sites in terms of $\delta^{18}O$ is odd considering hypothetical expectations. At less humid sites stomatal conductance should be reduced, causing a decrease in c_i/c_a and the observed enrichment in ^{13}C (Figure 3), but there should also be enhanced evaporative enrichment in ^{18}O for leaf water. However, differences in source water $\delta^{18}O$ (measured as stem water) will also affect cellulose $\delta^{18}O$ values. Expressing oxygen isotope ratios as deviations from stem water can clarify the effects of evaporative enrichment ($\delta^{18}O_{cellulose} - \delta^{18}O_{stem\ water}$). A plot of $\delta^{18}O_{cellulose} - \delta^{18}O_{stem\ water}$ versus $\delta^{13}C_{cellulose}$ (Figure 6) provides a way to relate the effects of humidity and water deficit on fractionation for both $\delta^{18}O$ and $\delta^{13}C$. Whereas the juniper and spruce sites were indistinguishable in terms of cellulose $\delta^{18}O$, the deviations in $\delta^{18}O_{stem\ water}$ at these two sites were distinct (Figure 6). All sites were significantly different ($p < 0.05$) in terms of $\delta^{18}O$ deviation.

[23] Site comparisons also benefit from using only a single portion of the ring. The pine and Douglas-fir sites have substantially more overlap on a $\delta^{18}O_{cellulose} - \delta^{18}O_{stem\ water}$ versus $\delta^{13}C_{cellulose}$ graph of earlywood alone or combined data (data not shown) than latewood alone (Figure 6). The range in $\delta^{18}O_{cellulose} - \delta^{18}O_{stem\ water}$ between sites (10‰) was similar to site differences in the oxygen isotopic composition of ecosystem respiration ($\delta^{18}O_R$) estimated at the same sites and at the same time as the tree rings sampled in this study were produced (2000 and 2001; refer to *Bowling et al.* [2003a] for $\delta^{18}O_R$ information) in contrast to minimal site variation in cellulose $\delta^{18}O$ (2‰, Figure 5).

3.3. Model Predictions Regarding Cellulose $\delta^{18}O$

[24] One of the striking features of this data set is the similarity in $\delta^{18}O$ values from sites with very different moisture availability (e.g., spruce and juniper sites). We collected environmental information to parameterize a mechanistic model [*Roden et al.*, 2000] to determine if this model was able to explain these observed tree ring $\delta^{18}O$ values. Daytime meteorological data used for modeling cellulose $\delta^{18}O$ for each site are presented in Figure 7. During the growing season, coastal sites were cooler and more humid than inland sites. Pine and juniper sites had similarly high vapor pressure deficits. The four sites had distinct source water $\delta^{18}O$ values (Figure 8). Mean stem water $\delta^{18}O$ values over an entire year were −6.0, −7.8, −13.6 and −10.8‰ (standard deviations ranged from 0.9 to 1.3‰) for the spruce, Douglas-fir, pine and juniper sites respectively. The pattern of source water variation relate to both the proximity to the ocean and subsequent rainout effects (a Rayleigh process) and temperature during precipitation events associated with elevation. Stem water $\delta^{18}O$ was more negative at the pine site than the juniper site, despite their relative distance from the coast (Figures 1 and 2). Atmospheric water vapor $\delta^{18}O$ was measured during the August 2001 field campaign. The mean atmospheric vapor $\delta^{18}O$ values measured regularly 12 times over a 24-hour period were −14.4, −14.1, −15.1 and −15.4‰ (standard deviations ranged from 0.8 to 1.2‰) for the spruce, Douglas-fir, pine and juniper sites respectively. Estimates of leaf diffusive conductance (stomatal + boundary layer) were derived from conductance/vapor pressure deficit relationships for coniferous trees [*Sanford and Jarvis*, 1986; *Grieu et al.*, 1988; *Kolb and Stone*, 2000].

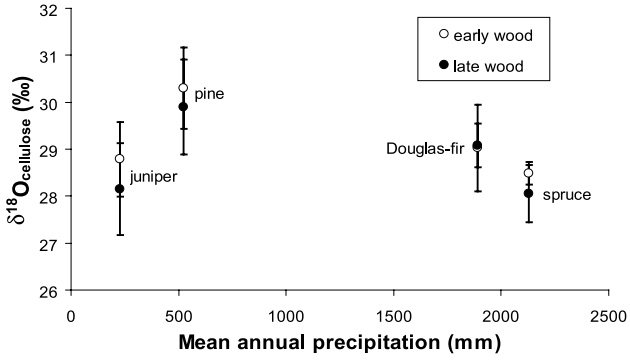

Figure 5. Mean oxygen isotopic composition of tree ring cellulose across the OTTER transect (± standard deviation, n = 12). Values for 2000 and 2001 are pooled since there was no significant difference ($p > 0.05$) between years for either latewood or earlywood. No correlations between cellulose $\delta^{18}O$ and precipitation were significant ($p > 0.05$).

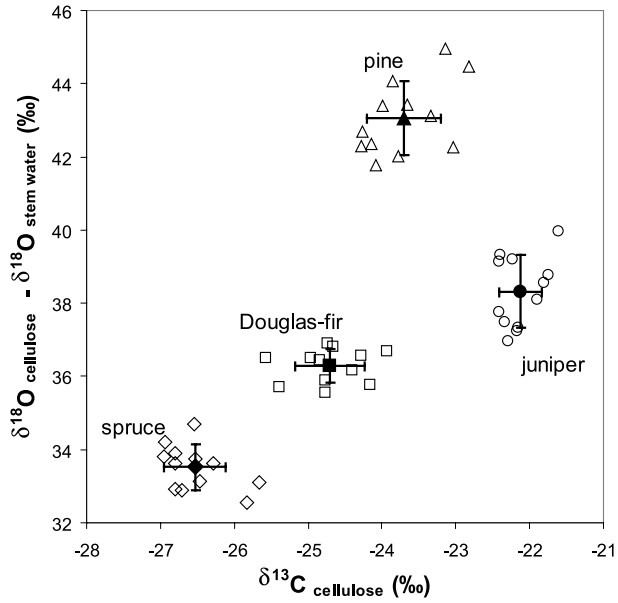

Figure 6. Deviation of oxygen isotopic composition of cellulose compared to stem water ($\delta^{18}O_{cellulose} - \delta^{18}O_{stem\ water}$) and the $\delta^{13}C$ for latewood cellulose from 2000 and 2001 at four sites along the OTTER transect. Symbols with the error bars (±1 standard deviation n = 12) within each grouping are mean values. Stem water was assumed to represent the source water used by the plant.

[25] The cellulose model accurately predicted that tree ring $\delta^{18}O$ should be less variable between sites (≈2‰, Figure 5) than between-site variation in either stem water (≈8‰, Figure 8) or leaf water (≈23‰, Figure 9b). The model underestimated both earlywood and latewood tree ring $\delta^{18}O$ for most of the sites except the juniper site (Figure 9a). However, the range of measured values and the range of model predictions generally overlapped. Bulk leaf water $\delta^{18}O$ was only measured during the August 2001 field campaign so no comparisons were possible with early season model predictions. The leaf water model [*Flanagan et al.*, 1991] predicted bulk leaf water quite well (when corrected for the fraction of unevaporated water) except for leaves at the juniper site. The range of possible leaf water values modeled was quite large (as much as 11‰) depending on the environmental parameters input, with humidity being the variable that had the largest impact on model outcomes.

4. Discussion

[26] A number of studies have linked the carbon isotopes in organic matter with moisture or humidity by examining precipitation transects. Some studies have looked at $\delta^{13}C$ in various plant tissues [*Guy et al.*, 1980; *Farquhar et al.*, 1989; *Stewart et al.*, 1995; *Schulze et al.*, 1996, 1998; *MacFarlane et al.*, 2004] across moisture gradients with results ranging from constant $\delta^{13}C$ across a transect to negative correlations with precipitation as in this study. For those studies that have utilized tree rings, relationships have been observed between the carbon isotopic composition and precipitation amount [*McCarrol and Pawellek*, 2001], potential transpiration [*Panek and Waring*, 1997], soil water deficit [*Porté and Loustau*, 2001] and plant water potential [*Warren et al.*, 2001].

[27] One issue with transects that span substantial distance is species turnover and inter-specific differences in physiology and function. Although surveys of tree ring $\delta^{13}C$ in various species of conifer can encompass the range of $\delta^{13}C$ found in this study (>8‰ [see *Warren et al.*, 2001]), comparisons are more valuable under common environmental conditions. *Marshall and Monserud* [1996] found no significant difference in tree ring $\delta^{13}C$ for three co-occurring conifers (*Pseudotsuga menziesii, Pinus ponderosa* and *Pinus monticola*). Carbon isotope discrimination values were similar between a juniper (*Juniperus osteosperma*) and pine (*Pinus edulis*) species growing in similar environments [*Williams and Ehleringer*, 1996]. *Panek and Waring* [1997] measured tree ring $\delta^{13}C$ of a single species (Douglas-fir) over a moisture gradient similar in magnitude to this study (>2000 mm). They observed significant site differences in $\delta^{13}C$ (>4‰) with drier sites more enriched in ^{13}C associated with humidity deficit. *Flanagan et al.* [1997] showed that carbon isotope discrimination was correlated with life form and that wood $\delta^{13}C$ was similar for two conifer species (*Picea mariana* and *Pinus banksiana*) growing at the same boreal forest sites. Although similar life forms (conifers only) were sampled in this study, we still

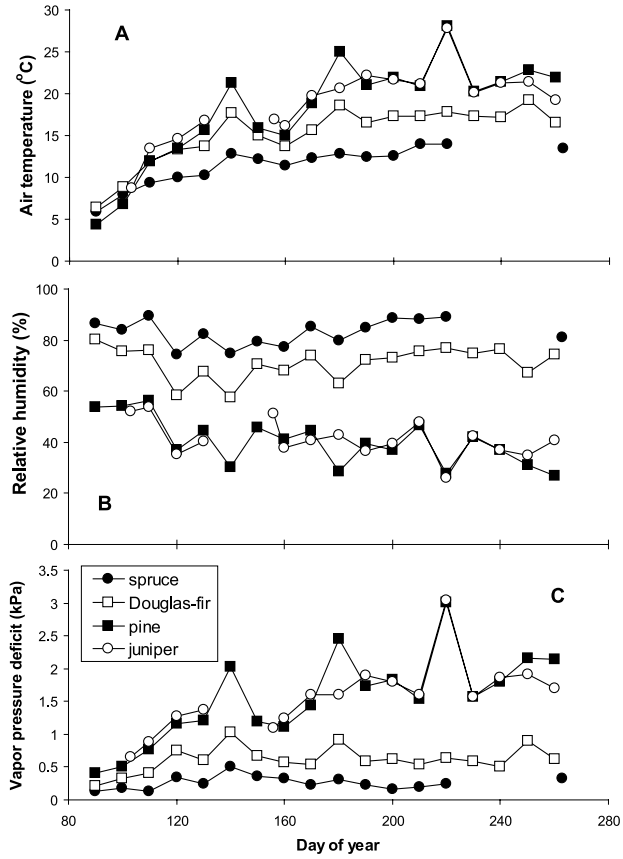

Figure 7. Growing season (a) air temperature, (b) relative humidity and (c) vapor pressure deficit for each site along the OTTER transect during 2001. Data are 10-day means of data collected hourly from 0800 local time to 1700 local time, and thus represent-daytime averages.

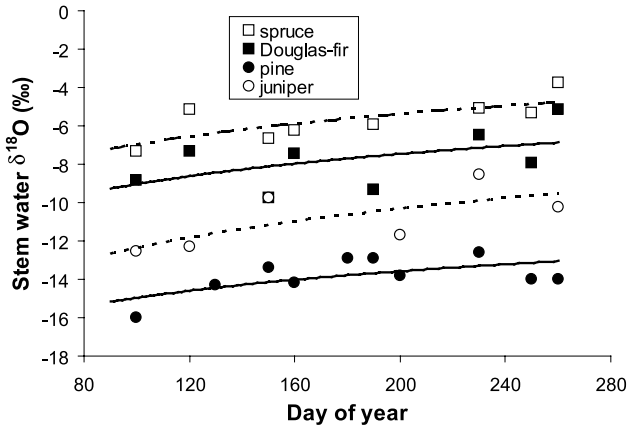

Figure 8. Growing season $\delta^{18}O$ of stem water measured for each site along the OTTER transect (2000 and 2001 measurements were pooled).

cannot partition all differences in $\delta^{13}C$ between sites as environmental.

[28] There was a striking lack of difference in cellulose $\delta^{13}C$ between the Douglas-fir and pine sites despite large difference in precipitation (Figure 3). Previous studies have established that ponderosa pine at the Metolius site access groundwater [*James et al.*, 2000; *Anthoni et al.*, 1999; *McDowell et al.*, 2004b]. Thus precipitation inputs at the pine site may not accurately reflect tree water status. The Douglas-fir site was a commercial plantation and was fertilized for stemwood production. Increased nitrogen inputs can increase $\delta^{13}C$ values owing to increased photosynthetic capacity [*Livingston et al.*, 1999; *MacFarlane et al.*, 2004]. The similarity in tree ring $\delta^{13}C$ for trees growing at the pine and Douglas-fir sites may be the result of both of these factors.

[29] This study was carried out concurrently with other projects [*Bowling et al.*, 2002, 2003a, 2003b; *McDowell et al.*, 2004a, 2004b] that describe the effect of water status on the variability in $\delta^{13}C$ and $\delta^{18}O$ of ecosystem respiration. *Bowling et al.* [2002] and *McDowell et al.* [2004b] found that sites with higher precipitation had more negative $\delta^{13}C$ values in plant and organic matter and that isotopic composition of respired CO_2 ($\delta^{13}C_R$) followed the same trends. *Pataki et al.* [2003] extended this result across a wide range of biomes. The $\delta^{13}C$ of tree ring cellulose, fine roots and ecosystem respiration were all strongly correlated with sun leaf $\delta^{13}C$ (Figure 4) indicating that current photosynthate may be the predominant substrate for respiratory flux within these systems. *Pataki et al.* [2003] also found that sun leaf $\delta^{13}C$ was correlated with the $\delta^{13}C$ of ecosystem respiration. Although the $\delta^{13}C$ of tree ring cellulose was more enriched by about 3‰ as compared to sun needles and fine roots, similar patterns across the OTTER transect were observed and the difference in $\delta^{13}C$ from the wettest to driest sites was comparable (4 to 5‰). These results highlight the importance of timescales of carbon storage in different ecosystem pools and their potential influence on atmospheric fluxes. In general, drier sites exhibited more enriched $\delta^{13}C$ in plant tissues, soil organic matter, ecosystem respiration [*Bowling et al.*, 2002; *McDowell et al.*, 2004b] and tree ring cellulose (this study [see also *Panek and Waring*, 1997]) than wetter sites. In our results, the cellulose $\delta^{13}C$ values were less variable than ecosystem $\delta^{13}C_R$ observations, which is likely due to temporal integration of isotopic inputs in organic matter. The tree ring data support the conclusions of *Bowling et al.* [2002] that differences in photosynthetic carbon isotope discrimination across the OTTER transect cause the observed variation in $\delta^{13}C$ of carbon stocks and fluxes.

[30] Few studies have linked the oxygen isotopic composition of tree ring cellulose with moisture or humidity. There were no distinct trends in $\delta^{18}O$ values of tree ring cellulose across the OTTER transect. Although meteoric and stem water $\delta^{18}O$ were more depleted at the arid sites than the wet sites (owing to rainout and temperature effects), evaporative enrichment associated with vapor pressure deficits overshadowed the $\delta^{18}O$ inputs from precipitation. In contrast to other studies [*Burk and Stuiver*, 1981;

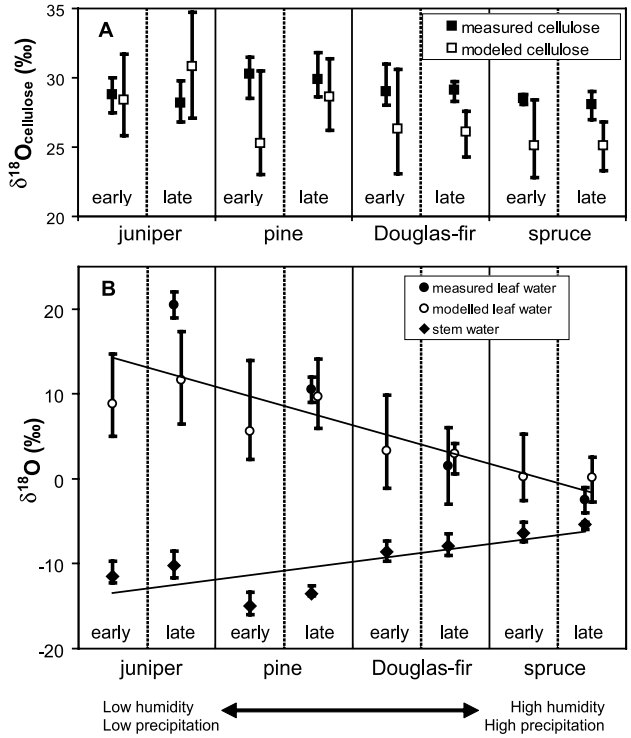

Figure 9. Measured (solid symbols) and modeled (open symbols) values of (a) tree ring cellulose and (b) measured and modeled August leaf water $\delta^{18}O$ and measured stem water $\delta^{18}O$, for each site along the OTTER transect. The data are split between early season ("early" denotes April to June environmental data used in the model and earlywood cellulose) and late season ("late" denotes July to September environmental data and latewood cellulose). Symbols are the mean of the measured values and the modeled value that represents the mean environmental conditions. Bars represent the range of measured values or the range of model predictions derived from the range of measured environmental conditions used to parameterize the model. The lines in Figure 9b were hand drawn and are not linear regressions since the *x* axis is categorical. They were included to provide a visual representation of trends across the transect and to relate our observations to Figure 2.

Roden and Ehleringer, 2000; *Barbour et al.*, 2001], there was a significant negative relationship ($p < 0.05$) between source water $\delta^{18}O$ and cellulose $\delta^{18}O$. In contrast to our study, *Saurer* [2003] and *Saurer et al.* [2002] found that the $\delta^{18}O$ in tree rings was positively correlated with precipitation amount as well as precipitation $\delta^{18}O$. In their study (across the northern tree line in Eurasia encompassing 150° of longitude), differences in tree ring $\delta^{18}O$ were related to variation in meteoric water $\delta^{18}O$ inputs associated with continental temperature variation [*Saurer et al.*, 2002]. Since precipitation amounts also decreased with continentality [*Saurer*, 2003], the positive correlation of tree ring $\delta^{18}O$ and precipitation likely resulted from the covariance of precipitation amount and meteoric water $\delta^{18}O$. A transect across the northern tree line may present minimal variation in vapor pressure deficit between sites, reducing the impact of humidity in modifying the relationship between source water $\delta^{18}O$ and tree ring $\delta^{18}O$ (in contrast to our results across the OTTER transect).

[31] Two questions arise regarding tree ring $\delta^{18}O$ observations from the pine and juniper sites. First, why was the stem water more depleted at the pine site than the juniper site? A trend of enriched cellulose $\delta^{18}O$ with aridity might have been stronger had the pine site had less negative source water $\delta^{18}O$. As stated above, the pine at the Metolius site had access to groundwater. This groundwater has a different isotopic composition from that expected from seasonal precipitation inputs. At no other site does it appear that trees have access to groundwater. Thus trees at the Metolius site are accessing a water source that does not fully reflect the expected inputs, leading to a more negative water source than available to juniper trees. Second, why did the $\delta^{18}O$ results indicate less evaporative enrichment for trees at the most arid site (juniper)? The $\delta^{18}O$ deviations from source water for juniper trees were much lower than for pine trees (Figure 6). We speculate that trees at the juniper site were active early in the growing season when soil moisture was high owing to snowmelt and temperatures were cool. This would lead to $\delta^{18}O$ values that indicate less evaporative enrichment than those in late summer. By later in the season the trees may be far less active owing to very dry soils. It is unknown when latewood is laid down for this species, but it could well represent a much different time of year than assumed for other species. Some studies [*Moore et al.*, 1999; *Law and Waring*, 1994; *Leffler et al.*, 2002] have demonstrated that water availability can severely limit leaf level gas exchange of juniper species, creating seasonal variation in carbon assimilation and transpiration. However, this interpretation must be tentative since the latewood $\delta^{13}C$ values for juniper trees do not likewise indicate reduced c_i/c_a values that would be indicative of reduced stomatal conductance.

[32] Our results do not support the prediction that tree ring cellulose $\delta^{18}O$ will necessarily increase with aridity because no significant correlation between precipitation and cellulose $\delta^{18}O$ was observed (Figure 5). However, when $\delta^{18}O$ values of cellulose are plotted as deviations from stem water $\delta^{18}O$ (Figure 6) the effects of water availability and humidity became apparent. *Bowling et al.* [2003a] measured the oxygen isotopes of CO_2 respired ($\delta^{18}O_R$) by these same ecosystems and found more enriched $\delta^{18}O_R$ values at the drier sites, indicating that evaporative enrichment associated with site vapor pressure deficits overshadowed the precipitation $\delta^{18}O$ input as a first order control on the $\delta^{18}O$ of ecosystem respiration. Our cellulose $\delta^{18}O$ data further confirm the importance of evaporative enrichment at these sites.

[33] Using $\delta^{18}O$ alone to infer site differences in water status may be problematic as shown in this study. Tree ring cellulose $\delta^{18}O$ for the juniper and spruce sites were indistinguishable, yet those sites differed dramatically in terms of water status and potential for evaporative enrichment. The similarities across sites are a result of counteracting influences of source water $\delta^{18}O$ and evaporative demand. However, using cellulose $\delta^{18}O$ deviations from stem water $\delta^{18}O$ captures the evaporative effect and allows for partitioning between sites (Figure 6). However, to derive useful climate information from ancient tree ring records, an independent estimate of stem water $\delta^{18}O$ would be required to calculate $\delta^{18}O_{cellulose} - \delta^{18}O_{stem\ water}$ from cellulose data, which is seldom available. Using a second isotope ($\delta^{13}C$) that is also influenced by vapor pressure deficit may allow better interpretation of $\delta^{18}O$ values recorded in tree rings. Each isotope provides different though related information regarding water status, and together, they provide a powerful data set to probe tree water status and its impact on carbon fluxes.

[34] Mechanistic models that predict cellulose $\delta^{18}O$ from environmental parameters [*Sternberg et al.*, 1986; *Yakir and DeNiro*, 1990; *Luo and Sternberg*, 1992; *Roden et al.*, 2000] have been successfully field tested on sites that differed in source water $\delta^{18}O$, humidity and temperature [*Roden and Ehleringer*, 2000]. The cellulose model used in the present study tended to underestimated $\delta^{18}O$ in tree ring cellulose (except for juniper trees), although some of the predictions based on mean environmental information were excellent. Our cellulose model was robust in predicting that differences in tree ring $\delta^{18}O$ between sites would be smaller than the differences between meteoric waters or leaf waters across the transect. Clearly, site differences, such as vapor pressure deficit, play a substantial role in modifying the primary $\delta^{18}O$ input of source water recorded in cellulose $\delta^{18}O$.

[35] Average environmental information may not necessarily be representative of conditions when photosynthesis is occurring and when cellulose is being constructed. Even though annual rings were subsampled, the slices represented an integrated sample over many weeks of metabolism. For example, the spruce and Douglas-fir sites experience high humidities and large amounts of precipitation, however, owing to low light intensities, very cloudy periods may be less important for photosynthesis than sunny and warm days. Periods of bright sunlight likely correspond to periods of lower humidity and thus greater evaporative enrichment in leaf water $\delta^{18}O$. Therefore mean environmental data may skew model predictions to periods of less photosynthetic activity and less evaporative enrichment. Our results support this hypothesis because the model underestimated spruce and Douglas-fir cellulose $\delta^{18}O$ (Figure 9). The ranges of possible outcomes (the error bars in Figure 9) were based on the range of measured environmental inputs and tended to overlap with observed values of cellulose $\delta^{18}O$. If sunny periods are more critical for carbon gain at the wet sites then the upper range of model predictions may

better represent the true environmental influence on cellulose $\delta^{18}O$.

[36] Model predictions of bulk leaf water $\delta^{18}O$ [*Craig and Gordon*, 1965; *Flanagan et al.*, 1991; *Roden and Ehleringer*, 1999a] were reasonably similar to observed values. This may imply that under-estimations of tree ring cellulose $\delta^{18}O$ were more a function of the cellulose model rather than the leaf water model. However, the leaf water observations were made during one field campaign in August of 2001, which may not necessarily be representative of integrated leaf water $\delta^{18}O$ over the entire summer period. No leaf waters were sampled during spring and, owing to limited sampling, broad conclusions regarding how well the leaf water model fits the data are tenuous at best. The *Craig and Gordon* [1965] [see also *Flanagan et al.*, 1991] model for evaporative enrichment assumes steady state conditions which may not apply during daily temperature and humidity fluctuations and thus a dynamic model may be more applicable [*Cernusak et al.*, 2002]. Unfortunately, the data to parameterize the *Cernusak et al.* [2002] leaf water model were unavailable (leaf water concentration, mole fraction of water vapor in the leaf intercellular spaces, and transpiration rates). In addition, using a dynamic model may not be critical since *Cernusak et al.* [2002] found that steady state models gave reasonable predictions during the daytime periods (when photosynthesis is occurring) and that long-lived, perennial plants may not be as influenced by diurnal fluctuations as crop plants. One interesting feature is how far off the model predictions for juniper leaves were compared to the measured August leaf water $\delta^{18}O$ values. Even with the depleted late-season leaf water $\delta^{18}O$ values predicted by the leaf water model, the cellulose model overestimated juniper latewood $\delta^{18}O$ by over 2‰. When the measured August leaf water $\delta^{18}O$ values were used instead, the model overestimated latewood cellulose $\delta^{18}O$ by 7‰. Although more leaf water sampling would be required to make any conclusions, this does support the idea, stated above, that latewood cellulose for juniper may be laid down much earlier in the season. August conditions may have been so dry that little or no wood was produced and the trees were simply involved in maintenance metabolism. At the other arid site (pine), latewood predictions of cellulose $\delta^{18}O$ were much better than for the juniper site. Since the pine trees on this site may have access to groundwater [*Anthoni et al.*, 1999; *McDowell et al.*, 2004b], late season environmental information may be more relevant for modeling leaf water and latewood cellulose $\delta^{18}O$ values than for juniper trees (Figure 9). *Law and Waring* [1994] found that drought and vapor pressure deficit were equally limiting to carbon uptake in juniper, while vapor pressure deficit had a larger effect than drought on seasonally integrated carbon uptake in pine. Model predictions would also be enhanced if reasonable assumptions could be made regarding what time of the year each portion of the ring is constructed and what carbon sources are utilized (stored versus current). For example, if we assume that latewood cellulose for juniper trees was produced from carbon sources produced earlier in the growing season, then model predictions would better match observations (data not shown). More research is needed that can provide information about tree ring construction for a variety of species.

[37] Isotope models are unlikely to be useful for climate reconstruction of ancient tree ring records since the variables needed to parameterize these models would seldom be available. However, as we test and confirm, using field-based and controlled experiments, that these models are robust in their predictive ability we learn more about the controls of $\delta^{18}O$ variation in tree ring records, which will enhance our interpretation of those records. This study demonstrates that useful information on site water balance can be gleaned from tree ring cellulose $\delta^{13}C$ and $\delta^{18}O$. Thus climate change events that cause shifts in site water balance could be studied by using long-term tree ring records. Many other studies [*Leavitt and Long*, 1991; *Loader and Switsur*, 1995; *Robertson et al.*, 1997; *Anderson et al.*, 1998; *Saurer*, 2003] have also demonstrated that stable isotope dendrochronology can be a powerful tool for climate analysis and reconstruction.

5. Conclusions and Implications

[38] Carbon isotopic composition of tree ring cellulose increased with aridity along the precipitation transect, supporting our hypothesis that differences in soil moisture and vapor pressure deficits between sites modify stomatal conductance, gas exchange and the $\delta^{13}C$ composition of organic matter. The $\delta^{13}C$ in tree ring cellulose varied in a similar pattern to ecosystem respiration $\delta^{13}C$. Minimal differences in tree ring $\delta^{18}O$ across sites indicate that site differences in source water $\delta^{18}O$ were overshadowed by vapor pressure deficit effects on evaporative enrichment. Thus historical records of cellulose $\delta^{18}O$ may provide useful proxy information regarding humidity and site water balance especially if combined with the complementary information located in the $\delta^{13}C$ of the same sample and if reasonable assumptions regarding source water $\delta^{18}O$ values can be made. A mechanistic model was capable of predicting the $\delta^{18}O$ in tree ring cellulose (to a first approximation) in field grown trees. These observations and model predictions demonstrate that environmental influences on evaporative enrichment can have profound effects on cellulose $\delta^{18}O$ even across a transect with substantial site differences in source water $\delta^{18}O$.

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