

Co-occurring species differ in tree-ring $\delta^{18}\text{O}$ trends

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Summary The stable oxygen isotope ratio ($\delta^{18}\text{O}$) of tree-ring cellulose is jointly determined by the $\delta^{18}\text{O}$ of xylem water, the $\delta^{18}\text{O}$ of atmospheric water vapor, the humidity of the atmosphere and perhaps by species-specific differences in leaf structure and function. Atmospheric humidity and the $\delta^{18}\text{O}$ of water vapor vary seasonally and annually, but if the canopy atmosphere is well mixed, atmospheric characteristics should be uniform among co-occurring trees. In contrast, xylem water $\delta^{18}\text{O}$ is determined by the $\delta^{18}\text{O}$ of water being drawn from the soil, which varies with depth. If co-occurring trees draw water from different soil depths, this soil-water $\delta^{18}\text{O}$ signal would be manifest as differences in $\delta^{18}\text{O}$ among the trees. We examined the variation in tree ring $\delta^{18}\text{O}$, over eight decades during the 20th Century, among three species co-occurring in natural forest stands of the northern Rocky Mountains in the USA. We sampled 10 Douglas-firs (*Pseudotsuga menziesii* (Mirb.) Franco var. *glauca*), 10 ponderosa pines (*Pinus ponderosa* Laws.) and seven western white pines (*Pinus monticola* Doug!). As expected, variation in atmospheric conditions was recorded in the $\delta^{18}\text{O}$ of the cellulose produced in a given year, but observed climatic correlations with $\delta^{18}\text{O}$ were weak. Significant correlations with June climate data included: daily maximum temperature ($r=0.29$), daily minimum temperature ($r=-0.25$), mean temperature ($r=0.20$), mean daily precipitation ($r=-0.54$), vapor pressure deficit ($r=0.32$) and solar radiation ($r=0.44$). Lagged effects were observed in Douglas-fir and western white pine. In these species, the $\delta^{18}\text{O}$ of a given annual ring was correlated with the $\delta^{18}\text{O}$ of the previous ring. Ponderosa pine showed no significant autocorrelation. Although the species means were correlated among years ($r=0.67$ to 0.76), ponderosa pine was consistently enriched in $\delta^{18}\text{O}$ relative to the other species; differences were close to 2‰ and they are steadily increasing. Relative to the mean for the three species, ponderosa pine is becoming steadily more enriched (-1.0‰). In contrast, Douglas-fir is being steadily depleted and western pine is intermediate, with an enrichment of 0.5‰. Because all trees were exposed to the same atmospheric conditions, the differences in $\delta^{18}\text{O}$ observed between species are likely due either to differences in the depth of water extraction or leaf function. If the former, presumably ponderosa pine has steadily taken up more water from near the soil surface and Douglas-fir has shifted uptake to a greater depth. If the latter,

we suggest the pronounced changes in leaf-water $\delta^{18}\text{O}$ are a result of changes in leaf structure and function with tree size and age.

Keywords: cellulose, climate, conifers, *Pinus monticola*, *Pinus ponderosa*, *Pseudotsuga menziesii*, stable oxygen isotope ratio, tree rings.

Introduction

Water in the environment contains varying amounts of oxygen-18 (^{18}O). The great majority of the oxygen in water (99.8%) exists as ^{16}O . However, a small fraction (0.2%), of the oxygen contains two extra neutrons: the stable ^{18}O isotope. Water molecules containing ^{18}O are slower to evaporate and diffuse and, conversely, quicker to condense and precipitate than those containing the lighter isotope, ^{16}O . For example, evaporation from cooler ocean water yields a smaller fraction of ^{18}O than evaporation from warmer water (Dansgaard 1964). The condensation of water due to orographic lifting tends to reduce the stable oxygen ratio ($^{18}\text{O}/^{16}\text{O}$) of the water remaining in the atmosphere; thus, progressively reducing $^{18}\text{O}/^{16}\text{O}$ as air masses move inland.

Geochemists first discovered geographic patterns in the $^{18}\text{O}/^{16}\text{O}$ of precipitation in the 1950s (Craig 1961). This variation was used to infer paleotemperatures beginning in the 1970s (e.g., Libby et al. 1976, McCarroll and Loader 2004 and references therein). Using tree rings, Saurer et al. (2002) found a large east-to-west $^{18}\text{O}/^{16}\text{O}$ gradient across northern Eurasia between the 19th and 20th centuries. They attributed the gradient to changes in seasonality of precipitation across the region. Another major source of variation is the temperature at which condensation occurs (Saurer 2003). These patterns have been described empirically (Bowen and Wilkinson 2002) by interpolating data from the Global Network for Isotopes in Precipitation (GNIP) database (<http://isohis.iaea.org>). Both empirical and mechanistic methods predict that winter precipitation will contain less ^{18}O than summer precipitation. If enriched summer rains displace depleted snowmelt downward, then vertical profiles of $^{18}\text{O}/^{16}\text{O}$ in soil water will occur (Rose et al. 2003).

Variation in $^{18}\text{O}/^{16}\text{O}$ can also result from physiological processes. Water is not fractionated as it enters roots and is carried

to the leaves (White et al. 1985); however, once in the leaves, the water is enriched in ^{18}O as a result of transpiration (White et al. 1985, Barbour et al. 2002). A freely evaporating surface tends to enrich leaf water in heavy isotopes because the lighter isotopes of hydrogen and oxygen in water vapor escape from liquid surfaces more readily than the isotopically heavy water molecules (Roden and Ehleringer 1999). The degree of enrichment is largely related to relative humidity. The enrichment of water influences the $^{18}\text{O}/^{16}\text{O}$ of the sugars produced in photosynthesis, which are then distributed throughout the tree. As the sugars are used for biosyntheses, some of the oxygen undergoes a final exchange, in situ, with the oxygen in water (Sternberg et al. 1986).

The mechanistic basis of the variation in $^{18}\text{O}/^{16}\text{O}$ of tree rings was outlined by Edwards and Fritz (1986) and subsequently refined (Roden and Ehleringer 2000, Roden et al. 2000). A general model that describes these processes (Roden et al. 2000) estimates the isotopic composition of leaf water using the Craig-Gordon model for evaporative enrichment (Craig and Gordon 1965), as modified by Flanagan et al. (1991). The model predicts the isotopic composition of leaf water as a function of relative humidity and the source water $^{18}\text{O}/^{16}\text{O}$. The ratio of $^{18}\text{O}/^{16}\text{O}$ in a plant sample is determined relative to the accepted standard by mass spectrometry and expressed in δ notation in per mil units:

$$\delta^{18}\text{O}_{\text{plant}} = \left(\frac{^{18}\text{O}/^{16}\text{O}_{\text{plant}}}{^{18}\text{O}/^{16}\text{O}_{\text{standard}}} - 1 \right) \times 1000 \quad (1)$$

where $\delta^{18}\text{O}_{\text{plant}}$ is the ratio of ^{18}O to ^{16}O in the plant tissue (Craig 1961) expressed relative to the Vienna standard mean ocean water (VSMOW) (Copier 1995).

The leaf water data are then combined with the xylem water data, based on empirical estimates of biochemical fractionation and proportional oxygen exchange:

$$\delta^{18}\text{O}_{\text{cx}} = f_0 \left(\delta^{18}\text{O}_{\text{wx}} + s_0 \right) + (1 - f_0) \left(\delta^{18}\text{O}_{\text{wl}} + c_0 \right) \quad (2)$$

where $\delta^{18}\text{O}_{\text{cx}}$ is the oxygen isotope composition of xylem cellulose, f_0 is the fraction of the oxygen in sucrose that exchanges with xylem water, $\delta^{18}\text{O}_{\text{wx}}$ is the oxygen isotope composition of xylem water, $\delta^{18}\text{O}_{\text{wl}}$ is the fractionation due to cellulose synthesis and $\delta^{18}\text{O}_{\text{wl}}$ is the isotopic composition of leaf water. According to Sternberg et al. (1986), s_0 is +27‰ and according to Roden et al. (2000), f_0 for oxygen in xylem cellulose is 0.42.

Recent work has suggested further complications in controls over $\delta^{18}\text{O}$ which might give rise to species differences. These differences, unexplained by the Craig-Gordon model, were first noted by Fdrstei (1986). Among the mechanisms proposed to explain these discrepancies is the back-diffusion of enriched water from the sites of evaporation in the leaf (the

P6clet effect; Farquhar and Lloyd 1993, Barbour et al. 2004). A second possible species difference is the degree to which leaves approach the steady-state conditions assumed by Equations 1 and 2 (White 1989, Cernusak et al. 2002).

Assuming that species differences are small for trees growing in the same forest stands, all fractionations and the isotopic composition of atmospheric water vapor are likely similar among sites and species and therefore, the only site and species-specific differences in the isotopic composition of plant tissue must arise from differences in the isotopic composition of xylem water. This assumption is based on the strong coupling between needle-leaved gymnosperm canopies and the surrounding atmosphere (Jarvis and McNaughton 1986). Differences in xylem water $2\text{H}/^1\text{H}$ and $\delta^{18}\text{O}$ have been related to different rooting depths in many species (e.g., White et al. 1985, Dawson 1993, Snyder and Williams et al. 2000, 2003). Hall (2005) described similar vertical profiles in late-summer $\delta^{18}\text{O}$ of soil water and species differences in depth of water uptake in our study area.

Our interest was in comparing tree ring $\delta^{18}\text{O}$ among species in the same forest stands over eight decades within the 20th Century. Our objective was to analyze the time series in $\delta^{18}\text{O}$ of tree-ring cellulose in a set of co-occurring trees of different species, with the goal of describing species differences and long-term trends. We also analyzed correlations with climate data and previous measurements of $\delta^{13}\text{C}$ on these same trees (Monserud and Marshall 2001). Finally, we analyzed patterns of temporal autocorrelation in $\delta^{18}\text{O}$ of these tree-ring records.

Materials and methods

Monserud and Marshall (2001) analyzed the carbon isotope ratios of tree ring cellulose deposited over the last 80 years in trees of western white pine (*Pinus monticola* Dougl.), ponderosa pine (*Pinus ponderosa* Laws.) and Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco var. *glauca*). The trees were growing together in naturally regenerated stands at the Priest River Experimental Forest (PREF) in northern Idaho, USA (116°50' W, 48°21' N). For most rings in their study, Monserud and Marshall (2001) collected an identical sample to serve as a laboratory backup in case of problems analyzing the first sample. We analyzed this backup sample for $\delta^{18}\text{O}$ in the cellulose.

The PREF is in the Selkirk Range, on the western flank of the Rocky Mountains, where the climate is intermediate between maritime and continental (Finklin 1983). One of the major reasons this series of research studies were carried out at PREF is that there are continuous weather station records from November 1911 (Finklin 1983). All stands in this study are located within 150 m a.s.l. of the weather station (at 800 m elevation). We used MTCLIM (Hungerford et al. 1989) to calculate climatic variables from the PREF weather records and to account for the minor differences in elevation between the individual stands and the weather station.

We focused on June data because earlywood is mostly produced in June. The beginning of earlywood production is in May, when buds break: In 1994, Monserud and Marshall

(1999) found that leaf expansion was completed by June 21 for Douglas-fir, July 6 for ponderosa pine and July 20 for western white pine. The end of leaf expansion has been correlated with the commencement of latewood formation (Larson 1969). Therefore, we expected strongest correlations with the June data.

The sampling protocol was the same as that described by Monserud and Marshall (2001; see their Figure 3 and Table 1). They sampled rings numbers 5-15 from the pith, using discs from every tenth whorl. This stair-step procedure resulted in a time series through each tree's lifetime, using rings from the same relative location in the upper portion of the crown throughout each tree's life. Each year was sampled once per tree. We intentionally sampled the innermost five rings only in the breast high disk, placing them at the start of the time series to minimize their effect on the rest of the series.

All trees, which were mature dominants or codominants with a normal, healthy appearance, were growing at altitudes between 800 and 950 m. The stands are the same as those studied by Marshall and Monserud (1996) and Monserud and Marshall (1999). Trees were destructively sampled in 1988 and 1989 by standard stem-analysis methods. Any discrepancy between ring counts and whorl counts was resolved by splitting the stem section down the pith and identifying terminal bud scars.

The original sampling protocol was to randomly sample 10 trees per species from a larger study of genetic and environmental variation at PREF. This resulted in 10 Douglas-fir, 10 Ponderosa pine and seven western white pine. Samples from the additional three western white pine trees were either unobtainable because of insufficient material or were lost in a sample preparation accident in the laboratory. Sample trees ranged from 60 to 85 years in age at breast height (bh; 1.37 m), with a mean of 76 years (Table 1). Tree diameter at bh ranged from 28 to 55 cm with a mean of 37 cm and total height ranged from 24 to 39 m with a mean of 31 m.

Each sample was dated and divided into earlywood and latewood based on distinct changes in color and resistance to peeling with a sharp blade (Monserud and Marshall 2001). Holocellulose was extracted from the earlywood according to the procedures of Leavitt and Danzer (1992). Because earlywood represents the period of highest transpiration rates by the tree, and therefore contains information on the most important part of the year, and because we were primarily interested in the annual water budget of the tree, we concluded that earlywood would be most informative. No material was combined across trees or across annual rings.

Table 1. Summary of sampled tree sizes, ages and $\delta^{18}\text{O}$. Values in parentheses are standard deviations.

Species	<i>n</i>	Diameter (mm)	Age (year)	Height (m)	$\delta^{18}\text{O}$ (‰)
Douglas-fir	10	378 (84)	78 (6)	28.8 (4.0)	26.1 (1.1)
Ponderosa pine	10	396 (93)	79 (9)	32.3 (4.2)	27.4 (1.0)
Western white pine	7	334 (41)	68 (5)	34.1 (1.2)	25.8 (0.9)

Statistical analyses generally followed the approach of Monserud and Marshall (2001). A significance level of $\alpha = 0.05$ was used throughout. Long-term trends were first removed by simple polynomials. Residuals were then analyzed by an iterative three-stage procedure for fitting Autoregressive Moving Average (ARMA) models using Box-Jenkins techniques (Box and Jenkins 1970). The main tool is the sample autocorrelation function (ACF) which is a simple summary (usually a graph-the correlogram) of the correlation structure within the series at increasing lags (e.g., the lag 1 autocorrelation is the correlation between all observations that are 1 year apart; Monserud and Marshall 2001). These techniques are described by Monserud and Marshall (2001).

The primary difference in methods from Monserud and Marshall (2001) is in the laboratory analyses. We used online pyrolysis of cellulose in the absence of added oxygen and over a glassy carbon catalyst, which quantitatively generated carbon monoxide (CO) gas exclusively from the oxygen in the sample (Werner et al. 1996, Farquhar et al. 1997). The $\text{O}/^{16}\text{O}$ of the CO gas was analyzed with reference to a standard CO (Messer, Frankfurt am Main, Germany). We used two consecutive 1-m columns of 0.5 nm molecular-sieve (Costech, Valencia, CA) to separate CO (mass = 28) from N_2 (mass = 28) effectively.

Results

Time trends: species means

We analyzed the mean $\delta^{18}\text{O}$ time series for each species and the overall mean. A significant quadratic trend was found for Douglas-fir ($R^2 = 0.20$). It was flat early in the study period, but underwent an accelerating decrease through the last decades (Figure 1). No significant trend over the eight decades was found for ponderosa pine, western white pine or the all-species combined mean series. The individual-tree time trends were similar (not shown). Only one of the 10 individual-tree series for ponderosa pine and one of the seven for western white pine had a significant time trend. In both cases the variation removed was small, 8%. In contrast, seven of the 10 Douglas-fir series had a significant slope with respect to time. These slopes explained from 10 to 22% of the variation. The variation among individual trees is indicated by the standard errors calculated for the means of the 10 Douglas-fir trees, which averaged 0.45‰ (Figure 2).

Between-species correlation

The correlations between species in $\delta^{18}\text{O}$ were all positive and highly significant. The bivariate linear fit between ponderosa pine and Douglas-fir explained 46% of the variation (Figure 3), the fit between Douglas-fir and western white pine explained 45% of the variation (Figure 4) and the fit between ponderosa pine and western white pine explained 58% of the variation (Figure 5).

We also plotted the 1:1 line of perfect correlation in Figures 3-5 to determine the relative offsets in $\delta^{18}\text{O}$ between species.

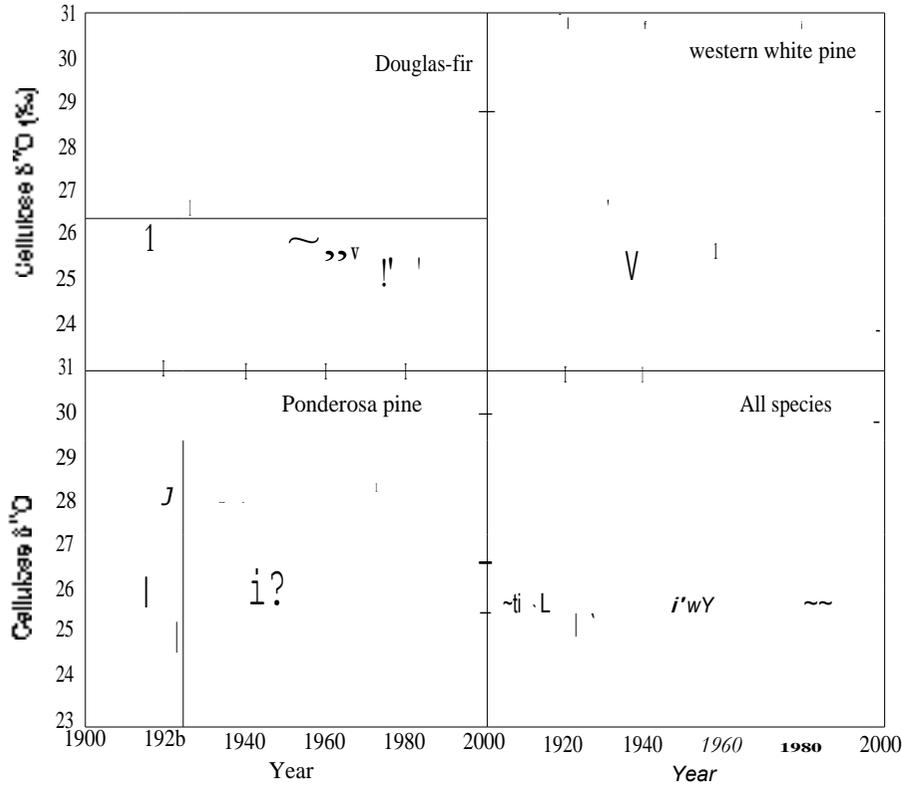


Figure 1. Mean cellulose $\delta^{15}\text{O}$ by species and averaged over all three species. Only Douglas-fir exhibits a significant time trend, which is quadratic.

An offset is apparent between ponderosa pine and Douglas-fir (Figure 3) and between ponderosa pine and western white pine (Figure 5). Ponderosa pine was always more enriched than the other species by about 2‰. No offset is apparent between Douglas-fir and western white pine, although the slope is not 1.0.

A useful way to view this between-species variation is to analyze the deviations in each year from the mean of all three

species (Figure 6). Douglas-fir deviations were strongly negative and they declined over time ($R^2 = 0.53$). The slope was -0.015‰ year^{-1} , which is a -1.5‰ change over 100 years relative to the all-species combined trend in $\delta^{18}\text{O}$ (Figure 6A). Ponderosa pine deviated strongly ($R^2 = 0.33$) over time, but in a positive direction (Figure 6C). The slope was 0.01‰ year^{-1} . Western white pine also deviated positively, but weakly ($R^2 = 0.09$, with a slope of 0.006‰ year^{-1} ; Figure 6B).

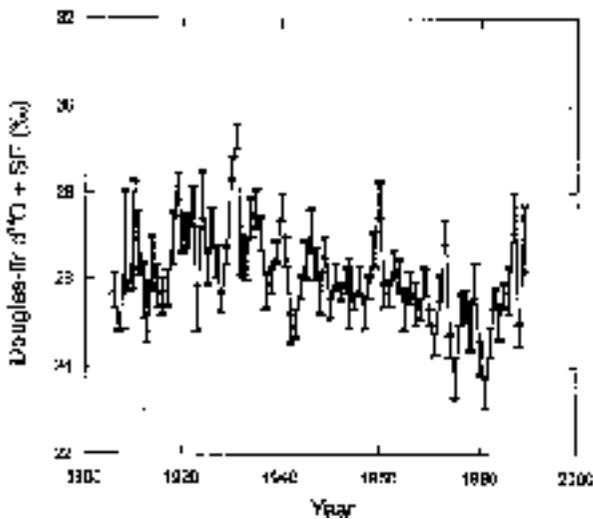


Figure 2. Mean $\delta^{18}\text{O}$ of Douglas-fir with standard errors (SE) calculated from ten trees.

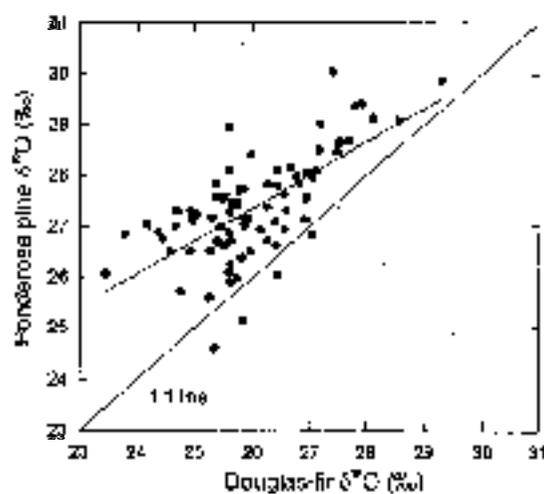


Figure 3. Correlations between ponderosa pine and Douglas-fir cellulose $\delta^{18}\text{O}$. Each value represents the mean of ten individuals per species for a particular year. The 1:1 line is shown for comparison.

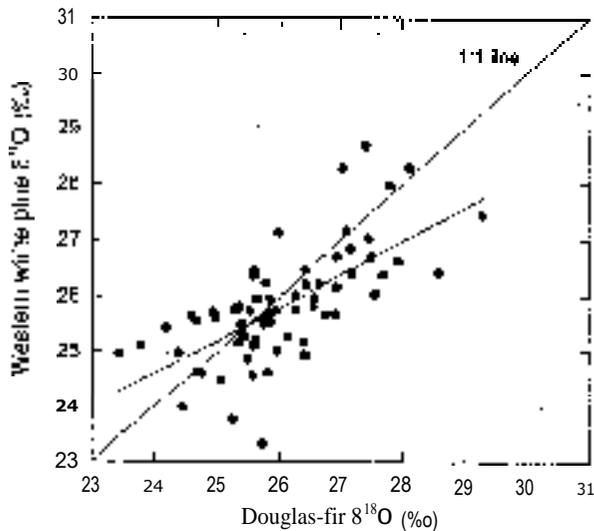


Figure 4. Correlations between western white pine and Douglas-fir cellulose $\delta^{18}\text{O}$. Each value represents a species mean for a particular year. The 1:1 line is shown for comparison.

When all three species deviations were plotted together (Figure 6D), the increasing deviations in $\delta^{18}\text{O}$ between Douglas-fir and ponderosa pine were evident.

Climatic correlation

We examined the bivariate relationships between each climatic variable (measured in June) and the all-species mean value of $\delta^{18}\text{O}$ over time.

Daily maximum temperature ($^{\circ}\text{C}$) was positively related to $\delta^{18}\text{O}$ (Figure 7A). Although significant, the relationship was weak ($R^2=0.09$).

Daily minimum temperature ($^{\circ}\text{C}$) was negatively related to $\delta^{18}\text{O}$ (Figure 7B). This relationship was significant, but weak ($R^2 = 0.06$).

Mean temperature ($^{\circ}\text{C}$) was positively related to $\delta^{18}\text{O}$ (Figure 7C), but the relationship was not significant ($R^2 = 0.04$).

Mean daily precipitation (cm day^{-1}) was negatively related to $\delta^{18}\text{O}$ (Figure 7D). This relationship was significant and fairly strong ($R^2 = 0.29$). When we examined species-specific variation in this relationship, we found that $\delta^{18}\text{O}$ was negatively related to daily precipitation in Douglas-fir ($R^2 = 0.18$), ponderosa pine ($R^2 = 0.26$), and western white pine ($R^2 = 0.25$) (Figure 8).

Vapor pressure deficit (Pa) was negatively related to $\delta^{18}\text{O}$ (Figure 7E). This relationship was significant, but weak ($R^2 = 0.10$).

Solar radiation (MJ m^{-2}) was significantly ($R^2 = 0.19$) positively related to $\delta^{18}\text{O}$ (Figure 7F).

Correlations with $\delta^{13}\text{C}$

We determined the correlation between $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ over time by species (Figure 9). Because the tree-ring earlywood samples for both the $\delta^{13}\text{C}$ (Monserud and Marshall 2001) and $\delta^{18}\text{O}$ were equal halves of the same sample material collected at the same time, we were able to determine correlations directly without introducing other sources of error. The correlation between $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ was positive for all three species (Figure 9). A linear relationship for both Douglas-fir and ponderosa explained 25% of the variation, whereas a linear relationship for western white pine explained 11%. The all-species combined correlation between $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ over time explained 15% of the variation (Figure 9).

Time series analysis

After removing the time trends, where they were significant, the residuals (or the original series if none of the time trends were significant) were analyzed for significant time lags using ARMA models. Six of the 10 Douglas-fir trees displayed significant autocorrelation. Four of the trees were best described by first-order models (a one-year lag), one by a second-order model (a two-year lag) and one by a third-order model. Only two of 10 ponderosa pine trees showed significant autocorrelation, one being second order and the other third order. The variation in $\delta^{18}\text{O}$ for the eight remaining ponderosa pine trees was random (white noise) over time. The autocorrelation memory in western white pine was more complex than in ponderosa pine, with four of seven trees displaying significant autocorrelation. Of these four trees, two were best described by first-order models and two by a third-order model. The variation in $\delta^{18}\text{O}$ for the remaining three was random white noise, with no year-to-year memory.

We then analyzed autocorrelation in the mean $\delta^{18}\text{O}$ time series for each species and for all three species combined. Because the only significant trend was in Douglas-fir ($R^2 = 0.20$), which was quadratic (Figure 1), a first-order autoregressive

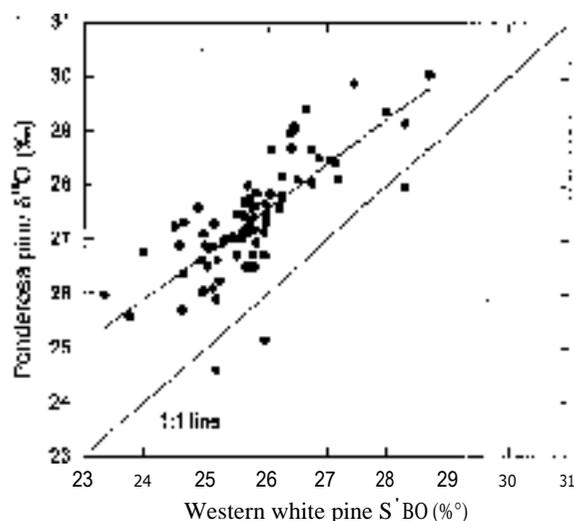


Figure 5. Correlations between western white pine and ponderosa pine cellulose $\delta^{18}\text{O}$. Each value represents a species mean for a particular year. The 1:1 line is shown for comparison.

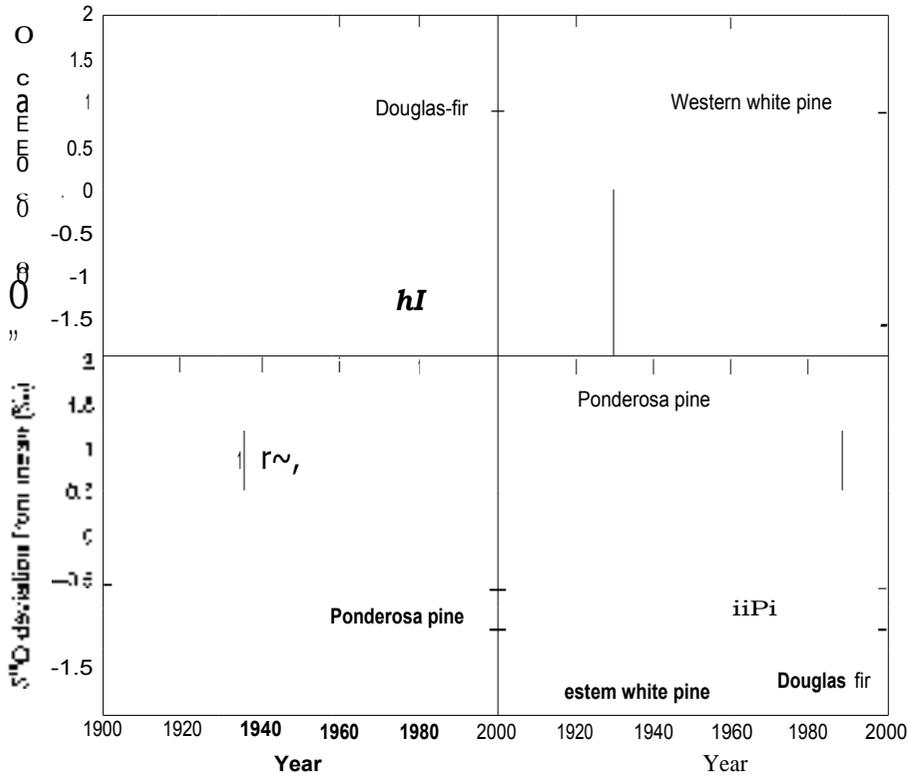


Figure 6. Species mean cellulose ^{18}O relative to the mean over all three species by year. The lower-right panel shows all three curves on the same axes for ease of comparison.

model best described the structures of the Douglas-fir, western white pine and the all-species combined series. In contrast, the mean ponderosa pine series was random white noise, with no year to year memory. Only the Douglas-fir and western white pine data are presented (Figure 10) because the overall pattern was driven by these species. The first-order autoregressive model, AR(1) is:

$$Z_t = \rho_1 Z_{t-1} + a_t,$$

where Z_t is the deviation at time t from the mean of an equally spaced stationary time series of length n , a_t is an independently distributed normal deviate with zero mean and constant variance σ^2 (a_t is white noise) and (ρ_1 is the first-order (lag 1) autoregressive parameter (Box and Jenkins 1.970)).

Discussion

We observed species differences in the 80-year time-series of ^{18}O in cellulose. Sternberg et al. (2003) point out that application of the Roden et al. (2000) model of cellulose isotopic composition, which provides a simple description of the mechanistic controls over ^{18}O without species effects, may contain an inherent ambiguity. A given value of ^{18}O results from three independent causes: the ^{18}O of soil water, the ^{18}O of atmospheric water vapor and the relative humidity. We resolved this ambiguity by selecting co-occurring trees from the same stands and sampling tree rings from the same years. Because the atmosphere is well mixed, we assumed that these

trees were growing in the same ^{18}O of atmospheric water vapor and relative humidity. Ignoring species effects, the only source of variation would be the ^{18}O of soil water, which is reflected in the ^{18}O of xylem water. Variation in ^{18}O of xylem water would most likely be a result of differences in the depth from which soil water is extracted. Species differences in vertical root deployment would result in differential uptake of water from various points in the vertical ^{18}O profile (Walker and Richardson 1991, Rose et al. 2003, Hall 2005).

Ponderosa pine was consistently more enriched in ^{18}O (by 1 to 2‰) than either Douglas-fir (Figure 3) or western white pine (Figure 5). Normally, more enriched water is found close to the surface owing to a combination of enriched summer rains and evaporative enrichment at the soil surface (Walker and Richardson 1991, Stratton et al. 2000). This pattern of shallow enrichment has been observed at the PREF in an August sampling of soil water (Hall 2005). The enrichment in ponderosa pine ^{18}O relative to the other species suggests that it is extracting water from nearer the surface than the other species, at least during June. Analyses of the depth of water uptake showed a different pattern in August, near the end of the summer drought. At that time, ponderosa pine appeared to be more deeply rooted than Douglas-fir and had a similar rooting depth as western white pine (Hall 2005). These results highlight the need for measurements of the vertical ^{18}O pattern of soil water in June.

The forestry literature concerning growing characteristics of our study species generally corresponds with our findings (Burns and Honkala 1990). The drought-tolerant ponderosa pine, which is often found on the driest sites that will support

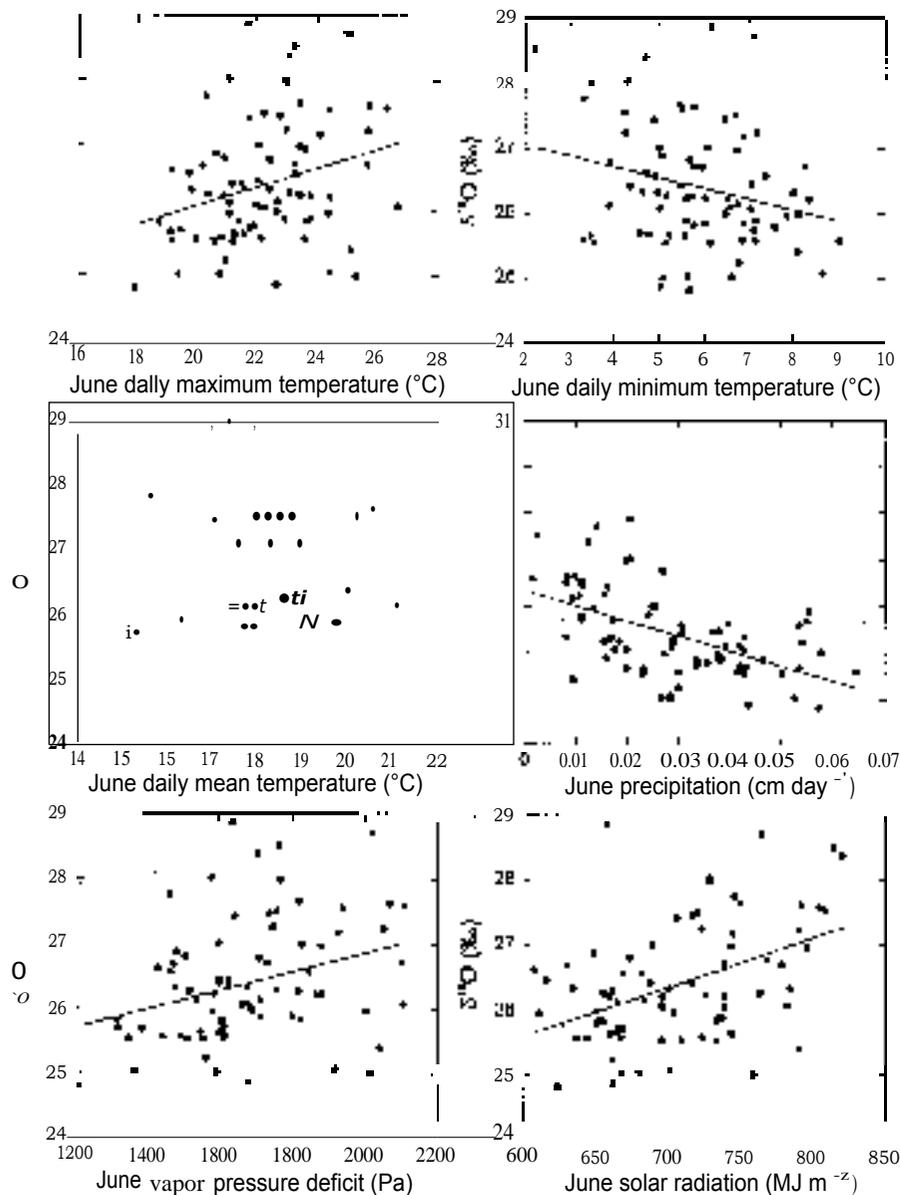


Figure 7. Correlations among the overall mean for cellulose and key climate variables.

trees, sends down a deep tap root enabling it to survive periods of severe drought and is often considered deeply rooted. However, this taproot is complemented by a shallow system of feeder roots that extract water from near the surface when conditions are mesic (Oliver and Ryker 1990). Perhaps these feeder roots are necessary because of the localization of nitrogen mineralization near the soil surface, which supports the high nutrient demands of the ponderosa pine canopy. This combination of deep and shallow rooting is an example of the dimorphic rooting pattern discussed by Ehleringer and Dawson (1992). In contrast, western white pine develops a deeper lateral root system, but with no deep tap root (Graham 1990). Rooting in Douglas-fir is rather similar to that in western white pine (Hermann and Lavender 1990), although on xeric sites, Douglas-fir can develop a tap root (Hermann and Lavender 1990).

Perhaps the least expected of our results was the pattern of species-specific variation in cellulose 5^{18}O over time. When compared with the overall mean (Figure 6), ponderosa pine became steadily more enriched (+1.0‰), whereas Douglas-fir became steadily depleted (-1.0‰); western white pine was intermediate, with an enrichment of 0.5‰. These species-specific changes occurred even though the mean 5^{18}O of the three species showed no trend (Figure 1). Thus, these species are diverging over time in their use of soil water, with ponderosa pine increasingly extracting enriched water, presumably from nearer the soil surface, and Douglas-fir increasingly extracting depleted water, presumably from greater depths, during the period when earlywood is formed. Because the 5^{18}O data describe the lives of the subject trees over eight decades, from seedling establishment through crown closure and self-thinning as they grew and competed for water and nutrients in the

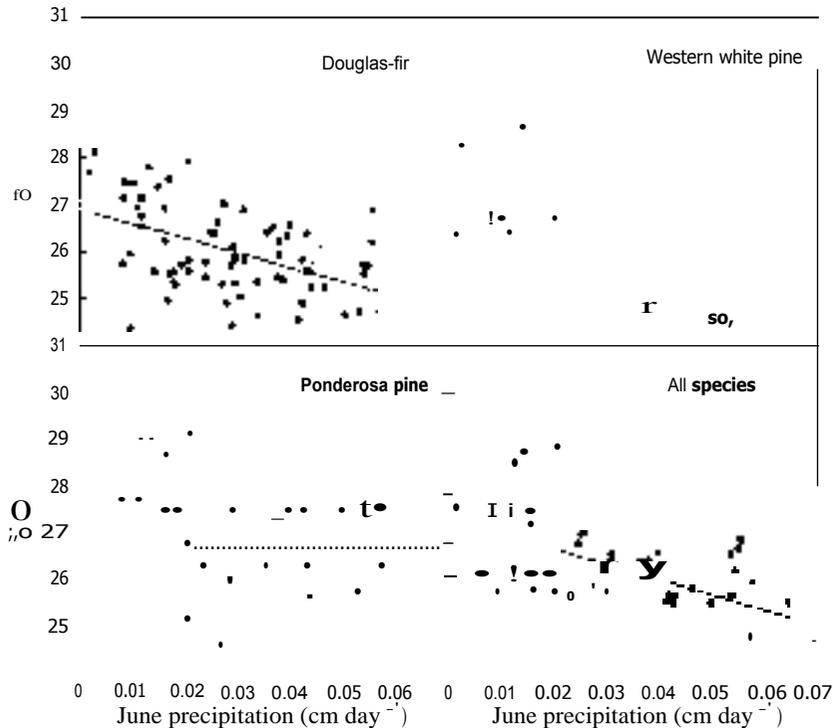


Figure 8. Correlations among June precipitation rates and mean cellulose ^{18}O by species. Each value represents the mean of seven to ten trees for a particular year.

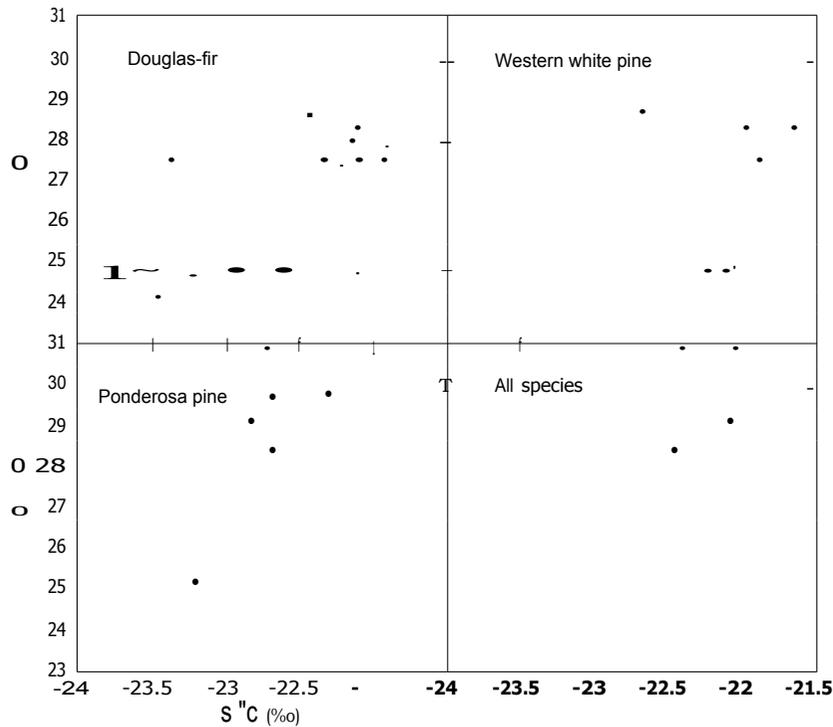


Figure 9. Correlations between cellulose ^{18}O and cellulose ^{13}C by species. Each value represents the mean of seven to ten trees for a particular year.

same natural stands, these species-specific shifts in ^{18}O reflect 80 years of belowground development in normal stands.

Continuous weather records at PREF are available since 1911; consequently, we were able to quantify the climatic van-

ation with MTCLIM (Hungerford et al. 1989). We focused attention on the June values of all climatic variables, which corresponds to the period of leaf expansion, rapid growth and earlywood formation for these species at PREF. We found that ^{18}O increased with increasing daily maximum temperature

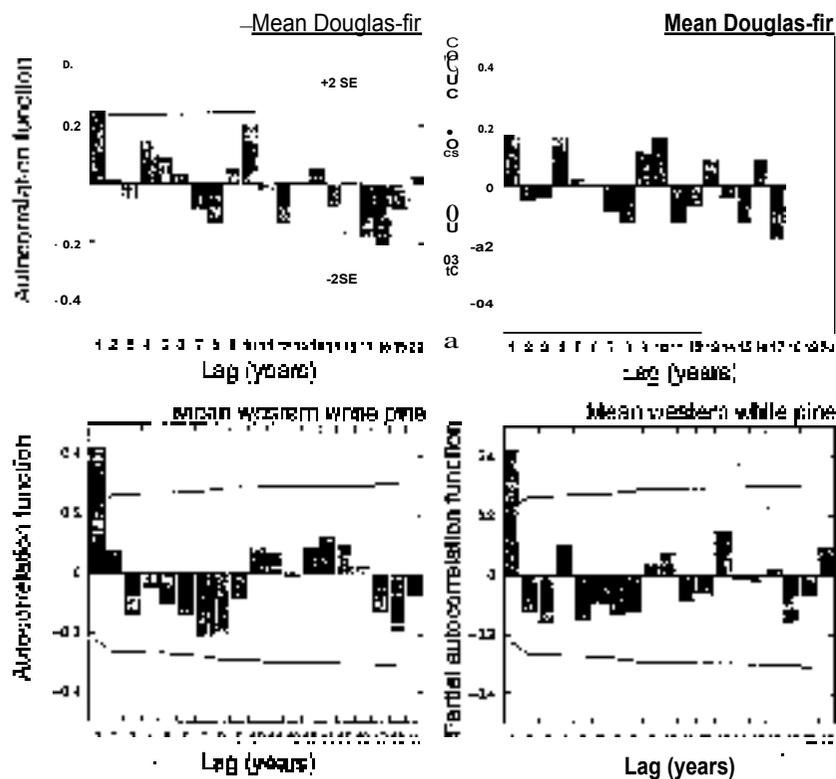


Figure 10. Autocorrelation functions and partial autocorrelation functions for the mean Douglas-fir and western white pine series. These are the two species for which significant one-year lags were detected. The thin lines represent \pm two standard errors, the approximate limit of statistical significance.

(Figure 7A) and decreased with increasing daily minimum temperature (Figure 7B). Both high maximum temperatures and low minimum temperatures would be associated with low humidity, which would increase cellulose ^{18}O values (Flanagan et al. 1991, Shu et al. 2005). The net effect of combining these extremes into the daily mean temperature is that the correlation disappears (Figure 7C).

The highest correlation with ^{18}O among the climatic variables was with June precipitation (Figure 7D). No species-specific differences in this negative trend were found (Figure 8). As monthly precipitation increases, humidity increases and evaporative ^{18}O enrichment would be inhibited. Thus far, Figure 7D is as expected. However, June summer rains are enriched in ^{18}O relative to winter precipitation (Flanagan and Ehleringer 1991), so one might expect that wetter June months would have higher ^{18}O values if surface water is extracted by the trees. Apparently, the reduction in evaporative enrichment during a wet June dominates over the enrichment of the soil water. The ^{18}O increased with increasing vapor pressure deficit (Figure 7E), as is expected with lower humidity (Flanagan and Ehleringer 1991). The ^{18}O also increased with solar radiation (Figure 7F), as expected because of the increased transpiration with greater leaf temperature and lower humidity.

These climatic correlations should be reflected in the stable carbon isotope ratio, $\delta^{13}\text{C}$ (Marshall and Monserud 1996, Monserud and Marshall 2001). Water stress, which may result from greater vapor pressure deficit, lower humidity, or drier soil, results in stomatal closure, which enriches the leaf inte-

rim with the heavier ^{13}C isotope, resulting in less negative $\delta^{13}\text{C}$ (DuPouey et al. 1993). We found a consistent positive correlation across all study species (Figure 9), as expected if stomata close in response to water stress. Saurer et al. (1997) also found linear relationships between ^{18}O and $\delta^{13}\text{C}$ for four temperate species in the Alps. The higher cellulose ^{18}O values of ponderosa pine relative to Douglas-fir and western white pine at a given $\delta^{13}\text{C}$ reflect the same species-specific responses observed in Figures 3-5.

We considered other potential causes of these patterns. First, the increase in leaf area index of the canopy might result in increased interception, which would tend to enrich the ^{18}O of throughfall and therefore of all species simultaneously. However, we observed no change in the overall mean ^{18}O over time. Second, competition with other species, which were not sampled, might influence the ^{18}O of the overstory study trees. In particular, these stands often initiate as brushfields, where the conifers must compete with deciduous angiosperm trees such as *Acer glabrum* Torr., *Alnus sinuata* Rydb. and several shrubs belonging to the family Rosaceae. However, such brush is suppressed as the canopies close and one might expect this process to yield a trend in the overall mean ^{18}O over time, but we saw no such trend. Another possible source of variation is seasonal and lifetime variation in the timing of earlywood production. Also, if photosynthesis were progressively shifted to the morning as trees grow taller, one might expect to find a record of lower humidity recorded as a shift toward more negative ^{18}O , over time. However, as no shift in overall ^{18}O was observed, we dismiss this source of variation as negligible.

Other possible causes of the species differences in long-term trends are related to changes in leaf structure and function. Leaf mass per area tends to increase with tree height in these stands as they grow and age (Marshall and Monserud 2003a, 2003b). Thicker leaves might lead to a longer effective path length from vascular tissue to evaporative surface, which would increase the Peclet number and reduce ^{18}O enrichment of leaf water (Wang et al. 1998, Barbour et al. 2004). This effect might be offset, at least partially, by lower transpiration rates from tall canopies (Ryan and Yoder 1997), which would lead to more enrichment. If species differed in the relative balance between the reduction in transpiration and the increase in path length, long term trends may result. A detailed analysis of the isotopic composition of xylem water, the phenology of leaf expansion and the diurnal timing of gas exchange would be necessary to answer these questions more conclusively.

Finally, variation in cambial phenology may contribute to the observed differences among species and their changes over time. If the water in the soil were substantially different or if there were a pronounced change in atmospheric humidity around the time of cambial activation, the result would be patterns such as described here. In a detailed study of cambial activation, growth commenced within about 10 days among various co-occurring conifer species in the Sierra Nevada Mountains of California (Royce and Barbour 2001). The commencement date varied considerably among years, but was synchronized in any given year to about two weeks after snowmelt. Conifers growing amidst competing shrubs (Royce and Barbour 2001) or other trees (Grotta et al. 2005) commenced growth a few days later than conifers growing in clearings. There was no clear change in commencement date as trees grew older and taller (Royce and Barbour 2001). Because we measured only the earlywood produced soon after the commencement of cambial divisions, any influence of the late-summer drought would not have been reflected in our data. The weakness of the species and age effects in the Royce and Barbour data (2001) argues against variation in cambial phenology as a major source of variation in ^{18}O , but it is difficult to draw definite conclusions without knowing how much ^{18}O might change over the couple of weeks when cambia are first activated, and it is difficult to know how well conditions in central California reflect those in Idaho.

The autocorrelation analysis yielded evidence of yet another sort of species difference. The mean ponderosa pine series behaved with no year-to-year memory of past values of ^{18}O over time. This corresponds to a species that relies strongly on water near the surface, the amount of which is essentially random from year to year. In contrast is the one-year memory of both Douglas-fir and western white pine, with an autocorrelation best described as first-order autoregressive. The previous year's value of ^{18}O explained a relatively small, but significant portion of the variation over eight decades in both species. The one-year memory in ^{18}O could be from the isotopically depleted deeper water, which could be stored in the soil profile for more than one growing season. Alternatively, it could result from the long lives (> 1 year) of roots produced at a given depth. The overall species mean is also best described by a

first-order autoregressive model, but this is presumably a reflection of the majority of trees comprising the overall mean being Douglas-fir and western white pine. The individual tree autocorrelation structures are much more complex for Douglas-fir and western white pine, with some individuals demonstrating significant second- and third-order memories. The temporal autocorrelation suggests that the ^{18}O of some trees, especially Douglas-firs, may be correlated with climate in the periods before the season in which the wood was deposited. Such an analysis would require a simultaneous fitting to climate data going back at least a year, determination of the probability that the autocorrelation was due to past weather and not past root distributions and consideration of the autocorrelation in the weather data—all of which was considered beyond the scope of this study and perhaps should wait until the mechanisms underlying the trends are identified.

Tree crowns rise, compete and differentiate over 80 years in closed-canopy stands (Muth and [BazZaz](#) 2003). This differentiation occurs both among and within species. What is difficult to imagine is a parallel, but hidden process beneath ground—the vertical differentiation in the root system and associated soil water extraction. The observed ^{18}O patterns may have resulted from shifts in the depth of soil water uptake with time. Alternatively, we may have detected shifts caused by changes in structure and function of the leaves themselves as they are elevated above the soil water supply.

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