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## **Research paper**

## Applying the dual-isotope conceptual model to interpret physiological trends under uncontrolled conditions

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The inter-relationships among  $\delta^{13}$ C and  $\delta^{18}$ O in tree ring cellulose and ring width have the potential to illuminate long-term physiological and environmental information in forest stands that have not been monitored. We examine how within-stand competition and environmental gradients affect ring widths and the stable isotopes of cellulose. We utilize a natural climate gradient across a catchment dominated by Douglas-fir and temporal changes in climate over an 8-year period. We apply a dual-isotope approach to infer physiological response of trees in differing crown dominance classes to temporal and spatial changes in environmental conditions using a qualitative conceptual model of the <sup>13</sup>C–<sup>18</sup>O relationship and by normalizing the data to minimize other variance. The  $\delta^{13}$ C and  $\delta^{18}$ O of cellulose were correlated with year-to-year variation in relative humidity and consistent with current isotope theory. Using a qualitative conceptual model of the <sup>13</sup>C–<sup>18</sup>O relationship and physiological knowledge about the species, we interpreted these changes as stomatal conductance responses to evaporative demand. Spatial variance between plots was not strong and seemed related to leaf nitrogen rather than any other environmental variable. Dominant trees responded to environmental gradients more consistently with current isotope theory as compared with other classes within the same stand. We found a correlation of stable isotopes with environmental variables is useful for assessing the impacts of environmental change over short time series and where growth varies only minimally with climate.

*Keywords*: crown dominance, Douglas-fir, relative humidity, stable isotopes, stomatal conductance, tree rings, water-use efficiency.

### Introduction

Stable isotope ratios in tree rings are powerful tools in ecological research because they indicate key environmental and physiological processes and record them over time. While several studies have clearly shown that the isotopic composition of tree rings can be a valuable source of information for the reconstruction of both plant carbon and water relations and environmental variability, most investigations to date have been based on independent analysis of  $\delta^{13}$ C or  $\delta^{18}$ O (McCarroll and Loader 2004). The biophysical mechanisms for fractionation of  $\delta^{13}$ C during photosynthesis have been understood for several decades (Farguhar and Sharkey 1982, Farguhar et al. 1989b, Ehleringer 1991, Ehleringer et al. 1993, Farquhar and Lloyd 1993, Lloyd and Farquhar 1994), and in recent years, the theory behind changes in stable oxygen isotopes ratio ( $\delta^{18}$ O) in plants has advanced significantly (Farquhar et al. 1998, Barbour 2007, Sternberg 2009, Kahmen et al. 2011). The examination of the inter-relationships among  $\delta^{13}$ C,  $\delta^{18}$ O and tree ring width has the potential to illuminate valuable physiological and environmental information (Saurer et al. 1997, Anderson et al. 1998, Scheidegger et al. 2000, Barbour 2007, Grams et al. 2007, Brooks and Coulombe 2009, Brooks and Mitchell 2011).

Carbon isotopes are frequently used to estimate an integrated measure of photosynthesis relative to stomatal conductance

 $(A/g_s)$ , which is a measure of the intrinsic water-use efficiency iWUE (Farquhar et al. 1989*a*, Lloyd and Farquhar 1994, Feng and Epstein 1995, Bert et al. 1997, Duquesnay et al. 1998). Recently, several studies have discussed the complicating factor of mesophyll conductance  $(g_m)$ , and identified that when  $g_m$ limits photosynthesis the connection between  $\delta^{13}$ C and iWUE weakens (Flexas et al. 2008, Seibt et al. 2008). Because  $g_m$  is relatively high and conservative in Douglas-fir needles (Warren et al. 2003*a*), measurements of  $\delta^{13}$ C in tree rings remains a powerful tool for understanding long-term water relations in forests (Francey and Farquhar 1982, Farquhar et al. 1989*b*, McNulty and Swank 1995, Bert et al. 1997, Brooks et al. 1998).

Unlike  $\delta^{13}$ C, the interpretation of  $\delta^{18}$ O largely remains uncertain because variation can be caused through several independent mechanisms (Epstein et al. 1976, Farquhar et al. 1998, Barbour 2007). The  $\delta^{18}$ O of plant material can be influenced by differences in  $\delta^{\rm 18}{\rm O}$  of source water, variation in  $\delta^{\rm 18}{\rm O}$  of water vapor in the air, the evaporative enrichment at sites of evaporation inside the leaf, and mixing of  $\delta^{18}$ O from evaporated and unevaporated source water during sugar and cellulose formation (Craig and Gordon 1965, Dongmann et al. 1974, Roden et al. 2000, Barbour 2007). With careful study design, some of these sources of variation can be eliminated. For example, if source water for trees in close proximity to each other is the same, then inter-tree variation in cellulose  $\delta^{\rm 18} O$  ( $\delta^{\rm 18} O_{\rm cell})$  is due to tree physiological processes. A growing number of studies have reported that when plants have the same water source, variation in  $\delta^{18}O_{cell}$  is strongly correlated with relative humidity (RH) and evaporative demand, which influences  $g_s$  (Flanagan et al. 1991, Barbour and Farquhar 2000, Barbour et al. 2000, 2004, Siegwolf et al. 2001, Farquhar et al. 2007, Kahmen et al. 2011, Roden and Farquhar 2012). Because <sup>18</sup>O enrichment in tree ring cellulose is influenced by RH (which also influences  $q_s$ ) and not by photosynthesis, the combined analysis of  $\delta^{13}$ C and  $\delta^{18}$ O has the potential to elucidate whether shifts in iWUE  $(A/g_s)$  are the result of shifts in the tree's photosynthetic capacity or shifts in  $g_s$ . Studies examining the relationship between  $\delta^{\rm 13}C$  and  $\delta^{\rm 18}O$  have largely used a qualitative approach that describes the long-term effects of environmental factors on leaf-level gas exchange (Saurer et al. 1997, Scheidegger et al. 2000, Brandes et al. 2006). Scheidegger et al. (2000) provided a conceptual model for deducing changes in gs and average maximum, light-saturated, net photosynthesis  $(A_{max})$  through examining the isotopic shifts in tree ring cellulose. Changes in environmental conditions over time or space can cause higher ( $\uparrow$ ), lower ( $\downarrow$ ) or similar ( $\approx$ )  $\delta^{13}$ C and  $\delta^{18}$ O values (Scheidegger et al. 2000, Saurer and Siegwolf 2007). The assumptions of the Scheidegger model are: (i) changes in  $\delta^{18}O_{cell}$  are primarily due to changes in leaf water enrichment caused by variation in air RH, because  $\delta^{18}$ O enrichment is dependent upon the ratio of the partial pressure of water vapor in the atmosphere  $(e_a)$  and the intercellular spaces in the leaf ( $e_i$ ) and  $e_a/e_i \approx RH$ , (ii) the  $\delta^{18}O$  of source water and of water vapor are the same among investigation periods, and (iii) an inverse relationship exists between  $\delta^{13}C_{cell}$ and [CO<sub>2</sub>] inside the stomatal cavity ( $c_i$ ). Based on these assumptions, the model predicts 'the most likely case' for the responses of  $g_s$  and  $A_{max}$  to a matrix of isotopic shifts (Scheidegger et al. 2000). Roden and Farquhar (2012) recently tested this conceptual model for applicability for tree rings, and they found relatively good agreement between measured gas-exchange data and model predictions from the tree ring isotopes.

Recently, more quantitative approaches have been employed to explore the relationship between  $\delta^{18}O_{cell}$  and  $g_s$ . Using  $\delta^{13}C$ and  $\delta^{18}$ O of leaf cellulose, Grams et al. (2007) demonstrated that under controlled environmental conditions, reductions in  $g_{\rm s}$  were associated with increases in  $\delta^{18}$ O of leaf cellulose in juvenile Fagus and Picea trees. The direct relationship between  $g_{\rm s}$  and  $\delta^{18}O_{\rm cell}$  was further inferred by Brooks and Coulombe (2009). By examining pre- and post-treatment  $\delta^{18}O_{cell}$  and  $\delta^{_{13}}C_{_{cell}}$  in contrast to control  $\delta^{_{18}}O_{_{cell}}$  and  $\delta^{_{13}}C_{_{cell}}$  in a Douglas-fir plantation, Brooks and Coulombe (2009) estimated that  $g_s$ was reduced by 30% in the dry, late growing season as a result of increased leaf area following nitrogen fertilization. In addition, Marshall and Monserud (2006) found consistent differences in  $\delta^{18}O_{cell}$  over several decades among competing tree species growing in the same environmental conditions. The authors hypothesized that the differences in  $\delta^{18}O_{cell}$  may have been a result of changes in leaf function (such as changing  $g_s$ ) with tree size and age, or that competing species used water from depths of the soil that had distinct isotopic differences. While it is clear that the interpretation of  $\delta^{\rm 18}{\rm O}_{\rm cell}$  and  $\delta^{13}C_{cell}$  works under highly controlled conditions, the technique still has potential for further development in less controlled settings.

To improve our understanding of the linkage between  $\delta^{18} O_{cell}$ and  $\delta^{13}C_{cell}$  in less controlled environments, it is important to understand how within-stand competition among trees, as well as environmental gradients, affects the sources and fractionation of C and O isotopes. For example,  $\delta^{13}C_{cell}$  has been found to increase with increasing vapor pressure difference and irradiance (Francey and Farguhar 1982, Farguhar et al. 1989a). In addition to temporal changes, both of these factors can vary across a small catchment, throughout the canopy profile, and among crown dominance classes, especially in species that form dense canopies. In addition, dominant trees within a stand tend to use more resources (i.e., water, light, nutrients) relative to other crown classes as forest stands develop, and in some cases dominant trees use these resources more efficiently than intermediate or suppressed trees (Oliver and Larson 1990, Smith and Long 2001, Binkley et al. 2002).

Here we apply a dual-isotope (<sup>13</sup>C and <sup>18</sup>O) approach to infer physiological response of trees to changing environmental

conditions in an uncontrolled setting. Our specific objectives were: (i) to separate the temporal and spatial influences of environmental gradients in tree-ring stable isotope analysis by using a normalization approach to reduce additional sources of variance, (ii) to compare our observed values of  $\delta^{13}C_{cell}$  and  $\delta^{18}O_{cell}$  to the qualitative conceptual model of the  $^{13}C^{-18}O$  relationship as presented in Scheidegger et al. (2000) and (iii) to examine how crown dominance might influence the  $\delta^{\rm 13}C_{\rm cell}$ and  $\delta^{18}O_{cell}$  response to environmental variation among years and among plots. We used natural variation in climate over 8 years and the micro-environment gradient across a steep catchment dominated by a single species (Pseudotsuga menziesii); the close proximity of the plots minimized isotopic variation in source water, water vapor and source CO<sub>2</sub>. We blocked for variation caused by time or space in order to explore all aspects of spatial and temporal variance.

## Materials and methods

#### Study site

The study area was a 96 ha watershed (Watershed One-WS1), located in the H.J. Andrews Experimental Forest (HJA) in the western Cascades of central Oregon, USA (44.2°N, 122.2°W). Elevations in WS1 range from 430 m at the gauging station to a maximum of 1010 m at the eastern ridge line. The HJA is a Long Term Ecological Research (LTER) site and has a meteorological data record from 1958 to the present. The HJA has a Mediterranean climate, with wet, mild winters and dry summers. Average annual rainfall is 2220 mm, of which ~80% falls between October and April (Rothacher et al. 1967). The watershed is predominately covered by mature Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco) replanted following clear-cut harvesting in the late 1960s and contains smaller components of western hemlock (Tsuga heterophylla (Raf.) Sarg.) and hardwood species (Moore et al. 2004). During our study, the maximum height of canopy ranged from ~22 to 31 m. A ridge-to-ridge transect of five plots (three south-facing and two north-facing) with a radius of 10 m was established in the spring of 2005. Two plots were located at the bottom of the slope and two were located near the ridge (~70 m higher in elevation relative to the bottom plots) with a third southfacing plot located midslope (~25 m higher in elevation relative to the bottom plots) to maximize the variation in local environmental conditions.

## Tree ring sampling and processing

Within each study plot, six trees were selected: two from each crown class—dominant, co-dominant and intermediate, determined by the diameter and height distribution of all trees within each plot. Crown class was defined by visual inspection of the canopy and by the diameter of each tree in relation to the diameter distributions of all trees within a given plot, where

dominant trees were  $\geq$  the 80th percentile and had canopies with crowns extending above the average crown cover, codominant trees were between the 60th and 80th percentile with canopies that formed the average crown cover of the plot, and intermediate trees were between the 40th and 60th percentile with crowns that were shorter than the dominant and co-dominant trees, but extended into the main crown cover. Trees were selected by establishing six rays uniformly distributed around the plot center and selecting a tree along each ray from within the randomly assigned dominance class. For each tree, diameter at breast height (DBH) and height (using a laser hypsometer; Forestry Suppliers, Jackson, MS, USA) were measured, and four 5 mm cores were obtained from the four cardinal directions near breast height. DBH was measured using a diameter tape while standing on the uphill side of tree at a height of 1.4 m.

We selected an 8-year period (2000-07) for isotopic analysis of the cores because these years contained a large range of year-to-year environmental variation and had the advantage of concurrent auxiliary data collected in 2005 and 2006 described below. Ring widths were measured along the entire core (~1974-2007). Cores were sanded to clearly observe earlywood and latewood boundaries. All cores were aged and cross-dated using marker rings to ensure accurate dating. We measured ring width using a tree-ring analysis system (WinDENDRO, Reg 2005c, Regent Instruments Inc., Quebec, Canada) attached to a digital scanner (Epson Expression, 10000 XL supplied and calibrated by Regent Instruments). Cores were scanned at 2400 dpi and measured for annual ring boundaries to 0.001 mm accuracy. Each tree-ring image was visually inspected and manually adjusted for accurate boundary detection. Basal area increment (BAI) was estimated using diameter measurements adjusted for bark thickness, and the average ring width of the four cores from each tree.

Recent studies have suggested that earlywood in tree rings is synthesized, at least partially, from stored photosynthates that were assimilated during the previous year, and as a result stable isotopes in earlywood may not be representative of current physiological processes; conversely, latewood is formed almost entirely from current photosynthate (Hill et al. 1995, Helle and Schleser 2004, McCarroll and Loader 2004, Kagawa et al. 2006a, 2006b, Offermann et al. 2011). As well, environmental conditions can change markedly between the early and late growing season in this Mediterranean climate which could obscure the relationship between isotopes and environmental variables if combined in one isotopic measurement per annual ring. For these reasons, we separated earlywood from latewood within each annual ring. Earlywood was distinguished from latewood by a step-change in color and wood density typical of Douglas-fir. After cores were measured and dated, each annual ring from each core from a

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given tree was cut into early- and latewood sections and combined into one early and one late sample per year (5 plots  $\times$  6 trees  $\times$  8 years  $\times$  2 wood densities). Samples were ground to a fine powder using a ball mill (Spex 5300, Metuchen, NJ, USA). Because the size of many samples was insufficient for isotopic analysis of individual trees, ground samples from the two trees within a given crown class (dominant, co-dominant, or intermediate) in the same plot were combined in equal amounts. All 240 samples (5 plots  $\times$  3 crown classes  $\times$  8 years  $\times$  2 wood densities) were extracted for  $\alpha$ -cellulose (Sternberg 1989, Leavitt and Danzer 1993).

#### Xylem water sampling

We sampled water from the xylem of trees within each study plot to determine if the  $\delta^{18}$ O of the source water varied spatially. Xylem water was assumed to reflect the isotopic composition of soil source water because trees do not fractionate water during uptake (White et al. 1985, Dawson 1993). At each plot, xylem samples were collected from suberized branches located in the sunlit, upper half of the canopy of three trees. Samples were collected every three weeks throughout the growing season of 2006. Xylem samples were collected in glass vials with polyseal cone inserts in the cap (VWR International, LLC, Batavia, IL, USA) and sealed to prevent evaporation. Water was extracted for 4 h from the samples using cryogenic vacuum distillation (Ehleringer et al. 2000).

#### Isotope analysis

Stable isotope composition of the  $\alpha$ -cellulose was measured on subsamples (~2.5 and 0.3 mg for <sup>13</sup>C and <sup>18</sup>O analysis, respectively) that were either combusted in an elemental analyzer (ECS 4010, Costech, Valencia, CA, USA) for  $\delta^{13}$ C, or pyrolized in a high-temperature conversion elemental analyzer (TC/EA, ThermoQuest/Finnigan, Bremen, Germany) for  $\delta^{18}$ O. The resulting gases were analyzed on an isotope ratio mass spectrometer (IRMS, Finnigan MAT Delta Plus XL or XP, Bremen, Germany) located at the Integrated Stable Isotope Research Facility at the Western Ecology Division of the EPA, Corvallis, Oregon. Xylem water samples were also analyzed for  $\delta^{18}$ O using the TC/EA and IRMS. All  $\delta^{13}$ C and  $\delta^{18}$ O values are expressed relative to their respective standard (PDB, V-SMOW) and expressed as ‰:

$$\delta = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1\right)$$

where *R* is the ratio of <sup>13</sup>C to <sup>12</sup>C atoms or <sup>18</sup>O to <sup>16</sup>O atoms of the sample or the standard. Measurement precision was better than 0.1 ‰ for  $\delta^{13}$ C and 0.25 ‰ for  $\delta^{18}$ O as determined from repeated measures of internal QC standards and from sample replicates.

### Environmental variables

Because environmental variables influence tree ring isotopes, we measured canopy air temperature (T) and RH in each study plot. At each plot, T and RH were measured at mid-canopy (HMP45c, Campbell Scientific Inc., Logan, UT, USA) and recorded by a datalogger (CR23X, Campbell Scientific Inc.) every 15 s and averaged over 15 min intervals. Plot-specific T and RH data were not available prior to 2005; therefore, we used long-term meteorological data sets to predict T and RH for 2000–05. Long-term data (T, RH and precipitation) were available from a nearby weather station (HJA Primary Meteorological Station (PRIMET)) located within 0.75 km of the study area. To predict T and RH for each plot for years prior to 2005, we used the linear relationship between simultaneous measurements made at PRIMET versus each plot individually during 2005 and 2006, and used the calculated linear relationships to predict plot level T and RH ( $R^2$  ranged from 0.92 to 0.99 for T, and 0.81 to 0.93 for RH). For each year, we divided plot-level environmental data into two time periods that were assumed to represent environmental conditions during earlywood and latewood growth conditions based upon observed seasonal declines in leaf-specific hydraulic conductance (Barnard 2009) and on past studies on the timing of latewood formation in Douglas-fir (Emmingham 1977, Vargas-Hernandez 1990, Beedlow et al. 2007). Earlywood environmental conditions were equal to the average T and RH for April through mid-July, and latewood conditions were defined as occurring from mid-July through the end of September each year. Only T and RH from 07:00 to 14:00 each day were used to calculate the average for each time period because canopylevel physiological processes (A and  $g_s$ ) are most active during this period. Transpiration consistently peaked in these plots at ~14:00 each day throughout the growing season (Graham et al. 2012).

#### Foliar nitrogen

Because nitrogen content is strongly correlated with foliar  $\delta^{13}$ C and photosynthetic capacity (Duursma and Marshall 2006), we examined the variability in foliar nitrogen content within our experimental plots. In August 2005 and 2006, we sampled current-year and 1-year-old foliage from the upper half of the canopy from three trees in each of the plots that were established in this study. All samples were ground to a fine powder using a mortar and pestle and then air dried. For each sample, up to 2 g of ground material was analyzed for carbon and nitrogen content. Analyses were performed using a CNS analyzer (CNS-2000 Macro Analyzer, Leco Corp., St. Joseph, MI, USA), which simultaneously determines carbon and nitrogen content of the solid samples.

#### Application of the Scheidegger conceptual model

We used the conceptual framework proposed by Scheidegger et al. (2000) to compare measured differences in  $\delta^{13}C_{cell}$  and

 $\delta^{18}O_{cell}$  to the theoretical predictions in  $g_s$  and  $A_{max}$  based on the two isotopes. In the case of isotope signatures recorded in tree rings, we consider shifts in the isotopes to represent a canopy integration of A and  $g_s$  (referred to as  $A_{INT}$  and  $g_{s-INT}$ hereafter, Figure 1). The integration incorporates the entire canopy weighted by relative photosynthetic rate and temporally integrates over either the early or late growing season. We use the model's predictions to infer physiological shifts between canopy positions and plots and over 8 years and to evaluate if those inferences match expectations based on current knowledge and observed environmental variation. Accordingly, we would interpret a positive correlation between  $\delta^{13}C_{cell}$  and  $\delta^{18}O_{cell}$  as a result of changing  $g_{s-INT}$ , but constant  $A_{\text{INT}}$  within our study trees either temporally or spatially. This relationship results from  $\delta^{\rm 18}{\rm O}_{\rm cell}$  becoming enriched with high RH causing  $g_{\text{s-INT}}$  to decline and  $\delta^{13}\text{C}_{\text{cell}}$  becoming enriched when  $A_{INT}/g_{s-INT}$  increases as  $g_{s-INT}$  declines. We interpret a negative relationship between  $\delta^{13}C_{_{cell}}$  and  $\delta^{18}O_{_{cell}}$  to suggest a change in integrated photosynthesis without a change in  $g_{s-INT}$ . In the case where no significant correlation exists, we examine the means and variance in  $\delta^{\rm 18}{\rm O}_{\rm cell}$  relative to  $\delta^{\rm 13}{\rm C}_{\rm cell}.$  For example, if  $\delta^{18}O_{cell}$  varies twice as much as  $\delta^{13}C_{cell}$ , and no correlation exists between them, we would interpret this as a constant  $A_{\rm INT}/g_{\rm s-INT}$  over a range of  $g_{\rm s-INT}$ . We used this framework to examine how physiology may have varied with respect (i) to dominance class, (ii) to changes over time, (iii) to differences between plots and (iv) to seasonal changes, and then relate those differences to the dominant environmental variable. For each analysis, we normalized the isotope data differently to eliminate other sources of variance. First, we examined how the dominance classes differed from a grand mean for each isotope for the early and late season. For each season, we calculated a mean for all years, all plots, and all crown classes (separate means for early and latewood), then subtracted that mean from the individual  $\delta^{13}C_{cell}$  or  $\delta^{18}O_{cell}$  values. We used



Figure 1. Conceptual application of the Scheidegger model (adapted from Scheidegger et al. 2000).

Pearson's correlation coefficients to quantify the relationship between  $\delta^{13}C_{cell}$  and  $\delta^{18}O_{cell}$  difference from the mean for each tree crown class.

Because RH varied temporally but not spatially among plots, we normalized isotope values for each crown class within each study plot with respect to time. For each crown class within each study plot, we calculated the mean values for all 8 years for each isotope (separate means for early- and latewood). We then subtracted each individual year's isotope value from the 8-year mean to obtain the 'anomalies' from the temporal baseline. We ranked the RH for each year (separate RH for each season) into three classes, high, medium and low RH, and assigned those classes to the samples. Finally, we examined the relationship between normalized  $\delta^{13}C_{cell}$  and  $\delta^{18}O_{cell}$  within the conceptual framework of the Scheidegger model to interpret possible physiological responses.

We had leaf nitrogen values for only 1 year (2006) in four of the five plots. For the south-facing midslope plot leaf nitrogen values are from the 2005 growing season. We assumed that although the absolute value of N content is likely to change from one year to the next within a single sample plot, the relative ranking among sample plots is likely to stay the same through time in a closed canopy forest such as our study site (Powers and Reynolds 1999, R. Powers, personal communication, 2009). Therefore, we normalized the data to minimize temporal variance and emphasize spatial variance. We calculated a mean  $\delta^{\rm 13}C_{\rm cell}$  and mean  $\delta^{\rm 18}O_{\rm cell}$  of the five plots for each year and for each dominance class (separate late- and earlywood means) and subtracted those from the appropriate  $\delta^{_{13}}\mathrm{C_{cell}}$  and  $\delta^{_{18}}\mathrm{O_{cell}}$  values. In this approach, the anomalies from the mean represent plot differences within a year. We ranked the study plots by their foliar N content from low to high and assigned the N-ranking to all tree ring samples from a given plot. We calculated the correlation between  $\delta^{13}C_{cell}$  difference from the mean and N rank. Within the context of the conceptual model, we anticipated that samples more enriched with <sup>13</sup>C would correspond with higher foliar N content.

Finally, we calculated the difference between late- and earlywood values of both  $\delta^{13}C_{cell}$  and  $\delta^{18}O_{cell}$  to examine if shifts in the isotopic composition might be explained by seasonal changes in water availability. We plotted the difference between late- and earlywood values of both isotopes against each other to visually examine the relationship within the Scheidegger framework. By using the Scheidegger model to interpret physiological shifts between seasons, we assume that the isotopic value of source water does not change seasonally. However, we discuss what the changes in source water isotopes would do as part of our interpretation.

#### Additional statistics

We performed repeated-measures analysis of variance to determine differences in  $\delta^{13}C_{cell},\,\delta^{18}O_{cell}$  and BAI with respect

to time and Sidak multiple comparison tests to determine differences among crown classes (Ott 1993). We examined the relationship among climate variables, BAI and shifts in isotopic composition by using Pearson product-moment correlation analysis. Because we were interested in the shifts in isotope values and not absolute values for correlations, we normalized isotope values for plot level differences by subtracting the mean isotope value for all 8 years within a given plot from each individual value. All statistics were performed using SPSS (Version 15.0, SPSS Inc., Chicago, IL, USA).

### Results

## Time series of $\delta^{13}C_{cell}$ , $\delta^{18}O_{cell}$ and BAI

The isotopic composition of earlywood samples was relatively constant over time for  $\delta^{13}C_{cell}$  (P = 0.75); however, all size classes were found to be significantly different from one another (P < 0.03 for all comparisons, Figure 2g). The  $\delta^{18}O_{cell}$ 

values were significantly different among years (Figure 2e, P < 0.01). Post hoc comparisons indicated year 2002 was significantly more enriched than year 2005 (P = 0.02), and dominant trees were significantly more enriched from intermediate trees. Similar to earlywood, the isotopic composition of latewood  $\delta^{13}C_{cell}$  did not significantly differ with respect to time (P = 0.07). Crown class was found to be significant (P = 0.03)with post hoc comparisons indicating dominant trees were significantly greater than intermediate trees (P = 0.03, Figure 2h). Latewood  $\delta^{18}O_{cell}$  differed with respect to both time and size class (P < 0.01 for both factors, Figure 2f). Dominant trees had the highest  $\delta^{18}O_{cell}$  values while co-dominant trees had the lowest  $\delta^{18}O_{cell}$ . Significant shifts in  $\delta^{18}O_{cell}$  occurred with increases from 2001 to 2002 and from 2004 to 2005, and decreases from 2003 to 2004. These changes were the inverse of shifts in mean RH, where  $\delta^{\rm 18}{\rm O}_{\rm cell}$  increased when RH declined and  $\delta^{\rm 18}{\rm O}_{\rm cell}$  decreased when RH increased (Figure 2d).



Figure 2. Temperature (*T*), relative humidity (RH),  $\delta^{18}O_{cell}$  and  $\delta^{13}C_{cell}$  through time. (a, b) Mean temperature for 07:00–14:00 h for early season (DOY 90-194) and late season (DOY 195-275), (c, d) mean relative humidity 07:00–14:00 h for early season (DOY 90-194) and late season (DOY 195-275), (e, f)  $\delta^{18}O_{cell}$  by crown class. Circles = dominant, squares = co-dominant, triangles = intermediate. (g, h)  $\delta^{13}C_{cell}$  by crown class with same symbols as above. Error bars equal the standard error of the means.



Figure 3. Annual BAI by crown class. Circles = dominant, squares = co-dominant, triangles = intermediate and May–October precipitation (PPT) is represented by the filled bars. Error bars equal the standard error of the means.

Basal area increment was consistent over time despite large year-to-year variation in summer precipitation (Figure 3). Within a given size class, the changes in BAI with respect to time did not differ (P = 0.08). However, as expected, the crown classes grew at significantly different rates (P < 0.01). Post hoc comparisons indicated that BAI of dominant trees was significantly greater than that of both co-dominant (P < 0.01) and intermediate trees (P < 0.01); however, no differences were detected between co-dominant and intermediate trees (P = 0.22).

The mean  $\delta^{18}$ O of the source water, as indicated by xylem water, did not vary significantly spatially during the 2005 and 2006 growing seasons (P = 0.14 and 0.87, respectively) but decreased later in the season corresponding to water isotopes deeper in the soil profile. Xylem water values averaged -10.4‰ (standard deviation = 1.24) for the 3 years. Xylem water values decreased 1.07‰ on average in 2005 and 2.06‰ on average in 2006 from the early season to the later season. In addition, we have strong evidence to believe that source water did not vary greatly on the inter-annual time scale. Annual precipitation water isotope values measured in Corvallis, OR have a standard deviation of 0.5‰ over 8 years with no significant seasonal variation (Brooks et al. 2012) and xylem water analysis from a nearby watershed averaged -10.4‰ in both 2004 and 2005 (Brooks et al. 2010). Based on these data, we assumed that  $\delta^{18}$ O of the source water did not vary spatially or year to year within our study.

# Correlations among environmental variables and $\delta^{\rm 13}{\rm C}_{\rm cell},$ $\delta^{\rm 18}{\rm O}_{\rm cell}$ and BAI

We correlated the temporal dynamics in tree ring normalized isotopes, and BAI with various environmental variables (Table 1). Earlywood isotopes were correlated with early season environmental variables (RH and *T*) and latewood isotopes were correlated with late season environmental variables as defined in the methods. Given the relatively small sample size ( $N \le 40$ ), significant correlations are identified at both the  $\alpha = 0.05$  and  $\alpha = 0.10$  level, but *P* values are also reported. Earlywood  $\delta^{13}C_{cell}$  for all crown classes increased with decreased early season RH.

This relationship was the strongest for co-dominant trees (r = -0.65), and weakest for intermediate trees (r = -0.31). Earlywood  $\delta^{18}O_{cell}$  also increased with decreased early season RH for dominant and co-dominant trees, but the relationship was not significant for intermediate trees. Earlywood  $\delta^{18}O_{cell}$  also tended to increase with decreased summer precipitation (PPT) for all crown classes. Data did not indicate that early season *T*, annual PPT or BAI were valuable indicators of shifts in isotopic composition in earlywood cellulose.

Latewood isotope values were also significantly correlated with RH with the exception of intermediate trees (Table 1). For the dominant and co-dominant crown classes, both  $\delta^{13}C_{cell}$  and  $\delta^{18}O_{cell}$  increased with decreased late season RH and decreased summer PPT. In addition,  $\delta^{13}C_{cell}$  increased with increased late season T for dominant and co-dominant trees (P < 0.01 and 0.08, respectively). We did not find any isotopic value or environmental variable with the exception of late season T to be significantly related to BAI, indicating that stable isotope values in tree ring cellulose were more sensitive to environmental variables than annual growth.

### Conceptual model of $\delta^{13}C_{cell}$ and $\delta^{18}O_{cell}$ relationships

We applied the conceptual model developed by Scheidegger et al. (2000) to estimate the potential physiological changes that have occurred with crown class differences, between plots and over time (Figure 1). When we compare the deviations in our isotope data to the overall mean for each dominance class and season,  $\delta^{13}C_{cell}$  tended to increase with increased  $\delta^{18} O_{cell}$  for both earlywood and latewood samples (but not always significantly) across all crown classes (Figure 4). In earlywood, co-dominant trees were significantly higher than the other crown classes in  $\delta^{13}C_{cell}$  (*P* = 0.03), but similar in  $\delta^{18}O_{cell}$  indicating higher  $A_{INT}$  according to the model. Dominant trees were significantly higher than intermediate trees in  $\delta^{18}O_{cell}$  (P < 0.01) indicating relative reduction in both  $A_{\rm INT}$  and  $g_{\rm s-INT}$ . In latewood, dominant trees had the highest  $\delta^{18} {
m O}_{
m cell}$  (P < 0.04) of all crown classes and higher  $\delta^{13} {
m C}_{
m cell}$ (P = 0.02) than the intermediate trees indicating that stomatal conductance was reduced according to the model interpretation. In contrast, co-dominant trees were significantly lower in  $\delta^{18}O_{cell}$  (P = 0.01) than other crown classes indicating a potentially greater stomatal conductance. The correlations between isotopes for both earlywood and latewood were strongest among dominant trees (r = 0.43 and 0.53, respectively and P < 0.01 for both correlations, Figure 4a and d). The model interpretation is that the temporal and spatial variance within the dominant trees was largely related to variation in stomatal conductance. This positive correlation also occurs in the earlywood of intermediate trees (r = 0.39, P = 0.02), and the latewood of co-dominant trees (r = 0.38, P = 0.02). The correlations we observed between RH and both isotopes over time (Table 1) can also be further explored in the Scheidegger

		5	Earlywoo	q				Latewo	po			
			BAI	Annual PPT (mm)	Summer PPT (mm)	Т (°С)	RH (%)	BAI	Annual PPT (mm)	Summer PPT (mm)	Т (°С)	RH (%)
Dominant	$\delta^{13}C_{cell}$	r	-0.08	0.21	-0.04	0.25	-0.37	0.01	0.12	-0.53	0.47	-0.46
		٩	0.61	0.20	0.80	0.12	0.02	0.99	0.45	<0.01	<0.01	<0.01
		Z	40	40	40	40	40	40	40	40	40	40
	$\delta^{18}O_{cell}$	r	-0.03	0.22	-0.29	0.19	-0.51	0.10	0.11	-0.40	0.33	-0.43
		٩	0.85	0.17	0.07	0.25	<0.01	0.54	0.49	0.01	0.04	<0.01
		Z	39	39	39	39	39	40	40	40	40	40
	BAI	r		-0.03	-0.05	-0.06	0.28		-0.03	-0.05	0.08	0.12
		٩		0.86	0.77	0.74	0.08		0.86	0.77	0.64	0.46
		Z		40	40	40	40		40	40	40	40
Co-dominant	$\delta^{13}C_{cell}$	r	-0.01	0.10	-0.25	0.38	-0.65	0.03	-0.04	-0.41	0.28	-0.31
		٩	0.96	0.53	0.13	0.02	<0.01	0.87	0.79	0.01	0.08	0.05
		Z	38	38	38	38	38	40	40	40	40	40
	$\delta^{18}O_{cell}$	r	0.02	0.23	-0.27	0.06	-0.27	0.08	0.11	-0.39	0.14	-0.30
		٩	0.89	0.15	0.10	0.70	0.10	0.63	0.50	0.01	0.38	0.07
		Z	39	39	39	39	39	39	39	39	39	39
	BAI	r		-0.02	-0.05	0.17	0.14		-0.02	-0.05	0.16	0.07
		٩		0.93	0.75	0.31	0.39		0.93	0.75	0.32	0.68
		Z		40	40	40	40		40	40	40	40
Intermediate	$\delta^{13}C_{cell}$	r	0.01	0.02	-0.33	0.16	-0.31	0.01	0.16	-0.42	0.26	-0.21
		٩	0.95	0.92	0.05	0.35	0.06	0.96	0.32	0.01	0.11	0.20
		Z	37	37	37	37	37	40	40	40	40	40
	$\delta^{18}O_{cell}$	r	0.09	0.24	-0.31	0.16	-0.12	0.14	0.15	-0.13	0.11	-0.25
		٩	0.62	0.16	0.06	0.34	0.47	0.39	0.35	0.44	0.50	0.12
		Ζ	37	37	37	37	37	39	39	39	39	39
	BAI	r		-0.01	-0.05	0.09	0.02		-0.01	-0.05	0.34	-0.23
		٩		0.97	0.75	0.57	0.91		0.97	0.75	0.03	0.15
		Z		40	40	40	40		40	40	40	40

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Figure 4.  $\delta^{13}C_{cell}$  values minus the mean  $\delta^{13}C_{cell}$  values for all samples versus  $\delta^{18}O_{cell}$  values minus mean  $\delta^{18}O_{cell}$  for all samples for earlywood (a–c) and latewood (d–f). Circles, squares and triangles are dominant, co-dominant and intermediate crown classes, respectively. Solid symbols represent the mean of the data with standard error bars. Data were normalized to the grand mean of all the data (all years, all plots and crown classes, but separate means for early and latewood). Points in each quadrant reflect the corresponding  $A_{INT} - g_{s-INT}$  response as indicated by the arrows.

conceptual framework (Figure 5). Deviations from zero in this figure represent year-to-year variation from the temporal average of each crown class for early- and latewood. For earlywood of all size classes, The  $\delta^{18}O_{cell}$  range was 1.26, 0.49, and 1.41‰ greater than the  $\delta^{13}C_{cell}$  range for dominant, codominant and intermediate trees, respectively. In latewood, range of variation from the mean is more equally distributed for both  $\delta^{\rm 18}{\rm O}_{\rm cell}$  and  $\delta^{\rm 13}{\rm C}_{\rm cell}$  (Figure 5) across all size classes, although the range of  $\delta^{\rm 18}{\rm O}_{\rm cell}$  was consistently greater than the range of  $\delta^{\rm 13}C_{\rm cell}$ . For latewood, the difference in range between  $\delta^{18}O_{cell}$  and  $\delta^{13}C_{cell}$  was 0.63, 1.24 and 1.16‰ for dominant, co-dominant and intermediate trees, respectively. Years with the highest RH had consistently lower  $\delta^{18}$ O and  $\delta^{13}$ C values than years with lower RH (Figures 2 and 5, Table 1). Within the Scheidegger conceptual model, it is assumed that as  $\delta^{18}$ O decreased, RH increased which caused  $g_{s-INT}$  to also increase. Therefore, Scheidegger et al. interpret greater changes in  $\delta^{18}$ O than in  $\delta^{13}$ C as  $A_{INT}$  and  $g_{s-INT}$  changing together over time resulting in constant iWUE over time.

Foliar nitrogen content in 2006 ranged from 0.88 to 1.11% for 1-year-old foliage and from 0.69 to 1.13% for current year foliage (Table 2). For both dominant and intermediate trees,  $\delta^{13}C_{cell}$  was significantly more enriched relative to the mean when foliar N was higher (Figure 6). Correlation coefficients for N versus  $\delta^{13}C_{cell}$  among dominant trees were 0.58 (P < 0.01) and 0.63 (P < 0.01) for earlywood and latewood, respectively. Intermediate-sized trees also had greater  $\delta^{13}C_{cell}$  with higher

foliar N content in both earlywood (r = 0.72, P < 0.01) and latewood (r = 0.70, P < 0.01). Surprisingly, in co-dominant trees,  $\delta^{13}C_{cell}$  was not related to foliar N content in earlywood (r = 0.10, P = 0.57) or latewood (r = -0.08, P = 0.63). In earlywood, deviations from the spatial mean for  $\delta^{18} \mathsf{O}_{\mathsf{cell}}$  were not related to foliar N content (P > 0.23, 0.51, and 0.49 for size classes). Coupling the  $\delta^{\rm 18}{\rm O}_{\rm cell}$  results with the  $\delta^{\rm 13}{\rm C}_{\rm cell}$ , the conceptual model indicates that  $A_{INT}$  was higher in high nitrogen trees in the early season. In latewood, deviations from the spatial mean for  $\delta^{18}O_{cell}$ were positively correlated with foliar N content for dominant (r = 0.63, P < 0.01) and intermediate (r = 0.49, P < 0.01) trees, but not for co-dominant trees (r = -0.15, P = 0.62). If changes in  $\delta^{
m 13}
m C$  relative to changes in  $\delta^{
m 18}
m O$  have a positive slope, Scheidegger et al. interpret this as an indication of  $g_{\text{s-INT}}$  varying more between plots than A<sub>INT</sub>. In earlywood, both dominant and intermediate trees had a significant positive slope (r = 0.43, P < 0.01; r = 0.43, P < 0.01, respectively). In latewood, all size classes had a significant positive slope (r = 0.55, P < 0.01; r = 0.44, P < 0.01; and r = 0.40, P = 0.01 for dominant, co-dominant and intermediate trees, respectively).

We calculated the difference between late- and earlywood values of both  $\delta^{13}C_{cell}$  and  $\delta^{18}O_{cell}$  to determine if seasonal shifts in the isotopic composition would be consistent with seasonal changes in  $g_s$  that others have noted for Douglas-fir in this Mediterranean climate (Bond and Kavanagh 1999, McDowell et al. 2002, Phillips et al. 2002, Winner et al. 2004, Barnard 2009). If declines in  $g_{s-INT}$  were responsible for isotopic



Figure 5.  $\delta^{13}C_{cell}$  minus temporal mean  $\delta^{13}C_{cell}$  versus  $\delta^{18}O_{cell}$  minus temporal mean  $\delta^{18}O_{cell}$  for earlywood (a–c) and latewood (d–f). Circles, squares and triangles are dominant, co-dominant and intermediate crown classes, respectively. Colors indicate relative humidity: black = highest RH years, white = lowest RH years. Data were normalized temporally such that deviations from zero represent year to year changes. Points in each quadrant reflect the corresponding  $A_{INT} - g_{s-INT}$  response as indicated by the arrows.

Table 2. Nitrogen composition	(%) of current	year (2006) foliage,	1-year-old foliage (	2005).
				. /

	Foliage N (SE)					
Plot	Current year	1-year-old	Average	Rank (low to high)		
South-facing ridge	0.69 (0.03)	0.88 (0.02)	0.79	1		
South-facing midslope <sup>1</sup>	0.94 (0.07)	0.94 (0.10)	0.94	3		
South-facing bottom	1.13 (0.10)	1.11 (0.02)	1.12	4		
North-facing bottom	0.81 (0.02)	0.97 (0.03)	0.89	2		
North-facing ridge	0.80 (0.05)	1.07 (0.02)	0.94	3		

<sup>1</sup>South-facing midslope plot values are from the 2005 growing season, where current year is 2005 and 1-year-old is 2004 foliage.

shifts between late- and earlywood, both isotopes should be more enriched in latewood relative to earlywood, but particularly  $\delta^{18}$ O. In the dominant crown class, nearly every sample of latewood  $\delta^{13}C_{cell}$  was enriched compared with the earlywood values, indicating that the dominant trees became more water-use efficient in the late season.

We note that earlywood can be a mixture of carbon fixed in the spring as well as stored carbohydrates from the previous fall and winter (Ogee et al. 2009), which would minimize the difference between late- and earlywood. In addition, 68% of the latewood  $\delta^{18}O_{cell}$  samples were enriched relative to the earlywood counterparts, indicating most dominant trees experienced a decline in stomatal conductance over the growing season. Because source water isotope values tended to decrease, not increase, through the growing season, changes in source water cannot explain our seasonal increase in  $\delta^{18}O_{cell}$ , but could explain why 32% of the latewood  $\delta^{18}O_{cell}$  samples were more depleted. A positive relationship existed between the two isotopes (r = 0.38, P = 0.02, Figure 7), indicating that water-use efficiency increased with decreasing stomatal conductance, which kept  $A_{INT}$  relatively constant through the season. Neither co-dominant nor intermediate trees showed a consistent increase in water-use efficiency through the season (r = -0.12, P = 0.52; r = -0.21, P = 0.22, respectively) which could be because of the confounding factors of changes in source water isotopic values from early to latewood production, and the use of stored carbohydrates in earlywood.

### Discussion

## The relationship among $\delta^{\rm 13}{\rm C}_{\rm cell},\,\delta^{\rm 18}{\rm O}_{\rm cell}$ and environmental variables

We found that  $\delta^{13}C_{cell}$  and  $\delta^{18}O_{cell}$  in tree ring chronologies can serve as proxies for a variety of environmental variables; however, the stable isotopes in dominant trees were the most responsive to environmental variables. Latewood  $\delta^{13}C_{cell}$  and



Figure 6.  $\delta^{13}C_{cell}$  minus spatial mean  $\delta^{13}C_{cell}$  versus  $\delta^{18}O_{cell}$  minus spatial mean  $\delta^{18}O_{cell}$  for earlywood (a–c) and latewood (d–f). Circles, squares and triangles are dominant, co-dominant and intermediate crown classes, respectively. Colors indicate rank of foliar nitrogen content (%): black = highest N, white = lowest N. Data were normalized spatially such that deviations from zero represent variation between plots. Points in each quadrant reflect the corresponding  $A_{INT}-g_{s-INT}$  response as indicated by the arrows.



Figure 7. The difference between latewood and earlywood  $\delta^{13}C_{cell}$  and  $\delta^{18}O_{cell}$  for dominant (a), co-dominant (b) and intermediate (c) crown classes. Points in each quadrant reflect the corresponding  $A_{INT} - g_{s-INT}$  response as indicated by the arrows.

 $\delta^{18}O_{cell}$  of dominant trees were significantly correlated with all environmental variables except for annual precipitation. Both earlywood and latewood  $\delta^{13}C_{cell}$  and  $\delta^{18}O_{cell}$  were negatively correlated with RH for dominant and co-dominant trees. These results are consistent with other studies, where increases in humidity result in reduced leaf evaporative enrichment of <sup>18</sup>O (Edwards et al. 2000, Barbour et al. 2002, Roden and Ehleringer 2007). Previous studies using  $\delta^{18}O$  of tree rings were focused on their use as a reconstructive tool for past temperature (Libby et al. 1976). In our study, temperature was significant, but only moderately correlated with latewood isotopes of dominant trees. Rebetez et al. (2003) noted that  $\delta^{18}O$  in tree rings was related to temperature only during the time period when the wood is formed. More recently, Kahmen et al. (2011) found that it was difficult to disentangle the independent effects of humidity and temperature in a tropical ecosystem, but by using the two metrics together in the form of vapor pressure deficit, a robust relationship could be determined between  $\delta^{18}$ O of cellulose and climate. Our results highlight the potential usefulness of stable isotopes in tree rings when applied to dendroclimatology, but suggest that dominant trees should be selected to obtain the best climate signals. Tree rings in this study demonstrated relatively uniform growth over time, and growth was not responsive to variation in environmental variables.

Dendrochronological studies in the Pacific Northwest have found Douglas-fir radial growth to be sensitive to both moisture and temperature, and the sensitivity can change with elevation (Little et al. 1995, Zhang et al. 1999, Lo et al. 2010). We did not find growth (BAI) to be correlated with either of these climate measures; however, it is possible that correlations may exist at finer seasonal scales or within a longer time series than were the focus of this study. Other authors have acknowledged the utility of stable isotopes with similar complacent growth responses (McNulty and Swank 1995, Robertson et al. 2008, Roden 2008). Because we did not find BAI to be significantly related to any of the environmental variables that we considered, we conclude that variability in  $\delta^{13}C_{cell}$  and  $\delta^{18}O_{cell}$  is more reliable than ring width as an indicator of interannual variability in climate in mature Douglas-fir trees.

## Interpreting $\delta^{13}C_{\mbox{\tiny cell}}$ and $\delta^{18}O_{\mbox{\tiny cell}}$ variation using the Scheidegger conceptual model

In this study, we relied on the conceptual model of Scheidegger et al. (2000) to interpret our dual-isotope results where changes in  $\delta^{18}O_{cell}$  are primarily due to changes in leaf water enrichment caused by variation in air humidity and changes in  $\delta^{\rm 13}C_{\rm cell}$  are related to plant water-use efficiency. Their model assumed that the  $\delta^{\rm 18}{\rm O}$  of source water and water vapor is the same among investigation periods and the relationship between  $\delta^{13}C_{cell}$  and  $c_i$  is negative. Based on these assumptions, Scheidegger et al. (2000) were able to predict 'the most likely case' for the responses of  $g_s$  and  $A_{max}$  for a matrix of potential isotopic changes (Figure 1). While the conceptual model was developed on leaves, its results have been shown also to be applicable to tree rings where the A and g are more integrated values of these processes over the entire canopy and over time (Roden and Farquhar 2012). The Scheidegger model provides the means for deducing changes in  $g_{s-INT}$  and  $A_{INT}$  through examining the isotopic shifts in tree ring cellulose through time.

We found a strong positive relationship between  $\delta^{13}C_{cell}$  and  $\delta^{\rm 18}O_{\rm cell}$  for dominant trees, and deduced from the conceptual model that A<sub>INT</sub> was relatively unchanged both spatially and temporally within our forest; the responses of  $\delta^{13}C_{cell}$  and  $\delta^{18}O_{cell}$  were interpreted as changes in  $g_{s-INT}$ . In comparisons of the  $\delta^{13}C_{cell} - \delta^{18}O_{cell}$  relationships between earlywood and latewood of dominant trees, latewood values fell within a quadrant that represents increased WUE ( $A_{INT}$  remain constant and  $g_{s-INT}$ declines), whereas earlywood samples were more uniformly distributed (Figure 4). This shift between earlywood and latewood (Figure 7) was likely due to seasonal reductions in  $g_s$ associated with our dry Mediterranean summers. In fact, Pypker et al. (2008) observed seasonal declines in canopy conductance in the same watershed during years of our study. This seasonal shift is consistent with our trends with annual RH, where years with low RH resulted in higher WUE for dominant trees (Figure 5). This pattern in the isotopes was observed despite the fact that xylem water became more depleted over the season reflecting deeper water sources. This shift and the potential use of stored carbohydrates for the production of earlywood would have lessened the differences we observed for dominant trees and could have masked the physiological effects in the lower crown classes.

In addition, we found that spatial differences in foliar N content supported the interpretations from the conceptual model. For dominant trees, our results indicated that spatial variations in  $\delta^{13}C_{cell}$  were strongly related to foliar N content (Figure 6). This is not surprising because it is well documented that high foliar N content generally increases  $A_{INT}$  (Field and Mooney 1986, Chapin et al. 2002, Duursma and Marshall 2006). Our study did not include temporal variations in foliar N; however, our results suggest that temporal measurements of foliar N may aid in interpreting the relationship between  $\delta^{13}C_{cell}$  and  $\delta^{18}O_{cell}$  within the framework of the conceptual model.

## On the importance of stand dominance in tree ring isotope research

In general, one would expect to find variations in the isotopic composition of tree rings among dominance classes due to vertical gradients in light,  $\delta^{13}C_{air}$  or RH (Elias et al. 1989, Buchmann et al. 1997, Hanba et al. 1997). One might also expect isotopic differences due to possible changes in hydraulic conductance with increasing tree size (Yoder et al. 1994, Koch et al. 2004, McDowell et al. 2011). Previous studies have shown that  $\delta^{13}$ C of leaves becomes more depleted lower in the canopy as light limits photosynthesis, thus decreasing  $A/q_s$  with canopy depth (Hanba et al. 1997, Duursma and Marshall 2006). Furthermore, vertical gradients in <sup>13</sup>C have also been linked to tree size, independently from light gradients (Marshall and Monserud 2003, Woodruff et al. 2004, McDowell et al. 2011). The observed decrease in  $\delta^{13}$ C of leaves has been attributed to increases in  $c_i$ and consequently, increased carbon isotope discrimination with decreases in light or increases in hydraulic conductance (Ehleringer et al. 1986, Farquhar et al. 1989a, Zimmerman and Ehleringer 1990, Hanba et al. 1997, Bond et al. 2007, McDowell et al. 2011). Consistent with prior studies, we observed intermediate sized trees growing under more light-limited conditions to be more depleted than dominant trees.

We observed the biggest difference between dominant trees and other crown classes when examining latewood  $\delta^{18}O_{cell}$ . Our results, along with previous studies, show a strong correlation between  $\delta^{18}O_{cell}$  and RH (Edwards et al. 2000, Barbour et al. 2002, Roden and Ehleringer 2007). If RH alone was responsible for the difference in  $\delta^{18}O_{cell}$  between dominant trees and other crown classes, we might infer that the canopy of dominant trees is exposed to wider variation in RH conditions than those of co-dominant or intermediate trees within the same stand. However, coniferous forests tend to be well coupled to the atmosphere and we do not expect large vertical gradients of RH to exist in our study plots, especially within the upper canopy during the daytime (Jarvis et al. 1976, Jarvis and McNaughton 1986, Monteith 1995). Nevertheless, co-dominant and intermediate trees may be less coupled to the bulk atmosphere and experience less variation in RH. We hypothesize that the difference in  $\delta^{18}O_{cell}$  between dominant trees and other crown classes may be due to more responsiveness in  $g_{s}$  $_{\rm INT}$  to environmental variation. Greater responsiveness of  $g_{\rm s-INT}$  in dominant trees could be due to wider variation in water stress of foliage at the top of the canopy. Stress-inducing mechanisms include increased leaf temperature, which in turn increases the leaf-to-air vapor pressure deficit and increased limitation to water transport as trees grow taller (Ryan and Yoder 1997, Martin et al. 1999, Niinemets et al. 2004). Our study does not have the required data to suggest if this mechanism might be responsible for a decline in  $g_{s-INT}$  in dominant trees. However, the difference in late- and earlywood  $\delta^{\rm 13}C_{\rm cell}$  and  $\delta^{\rm 18}O_{\rm cell}$  in dominant trees within the Scheidegger conceptual model suggests that seasonal reductions in  $g_{s-INT}$  are at least partially responsible for enrichment in  $\delta^{18}O_{cell}$ . Additional work examining the vertical profiles of  $\delta^{18}$ O in leaves with regard to  $g_{s-INT}$  is necessary to further our ability to interpret  $\delta^{18}O_{cell}$ .

## Precautions in applying the Scheidegger conceptual model

The Scheidegger conceptual model is highly useful for making generalized predictions of physiological responses from dual stable isotope data; however, it does rely on assumptions that may not always be true. For example, in our comparison of early- to latewood, xylem water became more depleted between the two time periods violating the assumption that the isotopic value of source water does not change. However, this source water effect should have created the opposite pattern in isotopes from what we observed, allowing us to still use the conceptual framework. The conceptual model interpretation also relies on the fact that stomata are responsive to changes in RH. Roden and Farquhar (2012) found seedling  $\delta^{18}$ O values to be highly responsive to RH, but the seedling gas-exchange measurements were not responsive to changes in RH. In our case, several studies have shown that stomata in mature Douglas-fir trees are responsive to vapor pressure difference and thus RH (Bond and Kavanagh 1999, McDowell et al. 2002, Phillips et al. 2002, Warren et al. 2003b, Moore et al. 2004, Unsworth et al. 2004, Winner et al. 2004, Bond et al. 2007). Nevertheless, caution should always be used when interpreting physiological responses from the conceptual model as RH is really driving changes in  $\delta^{18}O_{cell}$ .

Another complicating factor is that the model was originally developed on leaf-level data, so the application to more integrative tree rings may also present some problems. For example, the  $\delta^{18}O_{cell}$  values in tree rings represent a mixture of both evaporated leaf water and unevaporated xylem water,

whereas leaf  $\delta^{18}$ O contains only the leaf water signal (Roden et al. 2000, Barbour 2007). Thus, the scale of the  $\delta^{18}O$ responses will be more muted in tree rings compared to leaf cellulose. Investigating the biochemical fractionations and their timing that occur as oxygen and carbon are incorporated into wood cellulose is the focus of much recent research (Cernusak et al. 2009, Gessler et al. 2009, Ogee et al. 2009, Offermann et al. 2011, Roden and Farguhar 2012). Due to within-canopy variability of photosynthesis, stomatal conductance and leaf water enrichment, it is difficult to predict exactly what the isotopic shift from the leaves to the integrated wood cellulose will be. In addition, remobilization of starches from 1 year or one season to the next can also alter the isotopic signature recorded at a given point in the cellulose record (Allison et al. 1985, Jäggi et al. 2003, Farquhar et al. 2007, Ogee et al. 2009, Offermann et al. 2011). However, some studies indicate that remobilization and translocation only play a minor role in latewood development (Loader et al. 2003, Kagawa et al. 2005). These complex interactions can make the interpretation of tree ring isotopes extremely challenging in the absence of additional micrometeorological and isotopic source data. It is reasonable that these interactions may also be responsible in part for the lack of strong patterns observed in this study. Despite these challenges, we feel that the Scheidegger model remains a valuable tool in examining potential physiological responses through time when the assumptions of the model are met or exceptions can be documented. The isotopic theory linking oxygen and carbon isotopes in tree-ring cellulose to plant physiology has been extensively investigated and goes well beyond the conceptual model (Dupouey et al. 1993, Saurer et al. 1997, Duquesnay et al. 1998, Roden and Ehleringer 1999, Roden et al. 2000, Barbour et al. 2002, 2004, Barbour 2007, Sternberg 2009). Using the conceptual model is taking a step back from these explicit models and looking more generally at the patterns of isotopic change.

## Conclusion

Using a normalization approach to reduce sources of variance, we used natural environmental gradients both spatially in a steep catchment dominated by a single species, *P. menziesii*, and temporally over a series of years and seasonally to further our understanding of the relationships between  $\delta^{13}C_{cell}$  and  $\delta^{18}O_{cell}$ , annual ring widths, physiological processes and environmental variables. Using a qualitative conceptual model of the <sup>13</sup>C<sup>-18</sup>O relationship as presented in Scheidegger et al. (2000), we found evidence of  $\delta^{18}O_{cell}$  being related to changes in RH, and thus influencing  $g_s$  behavior over time. Spatial variance between plots was related to leaf nitrogen where plots with higher N values had higher  $A_{\rm INT}$  values, according to the model. We found that dominant trees behaved differently from sub-dominant trees within the same stand, and provide isotopic results that are most

consistent with current isotope theory. Stable isotopes in tree rings can be particularly useful for understanding physiological responses of forests to environmental change over short time series and where tree growth is relatively complacent with climate.

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## **Conflict of interest**

None declared.

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