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Interpreting tree responses to thinning and fertilization using tree-ring stable isotopes

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Summary

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- Carbon sequestration has focused renewed interest in understanding how forest management affects forest carbon gain over timescales of decades, and yet details of the physiological mechanisms over decades are often lacking for understanding long-term growth responses to management.
- Here, we examined tree-ring growth patterns and stable isotopes of cellulose ($\delta^{13}\text{C}_{\text{cell}}$ and $\delta^{18}\text{O}_{\text{cell}}$) in a thinning and fertilization controlled experiment where growth increased substantially in response to treatments to elucidate physiological data and to test the dual isotope approach for uses in other locations.
- $\delta^{13}\text{C}_{\text{cell}}$ and $\delta^{18}\text{O}_{\text{cell}}$ results indicated that fertilization caused an increase in intrinsic water-use efficiency through increases in photosynthesis (A) for the first 3 yr. The combination treatment caused a much larger increase in A and water-use efficiency. Only the thinning treatments showed consistent significant increases in $\delta^{18}\text{O}_{\text{cell}}$ above controls. Changes in canopy microclimate are the likely drivers for $\delta^{18}\text{O}_{\text{cell}}$ increases with decreases in relative humidity and increases in leaf temperature associated with thinning being the most probable causes.
- Tree-ring isotopic records, particularly $\delta^{13}\text{C}_{\text{cell}}$, remain a viable way to reconstruct long-term physiological mechanisms affecting tree carbon gain in response to management and climate fluctuations.

Introduction

Efforts to increase carbon sequestration have focused renewed interest on understanding how forest management affects forest carbon gain over timescales of decades. Two of the most common forest management tools are thinning and fertilization, and yet details of the physiological mechanisms responsible for carbon gain responses to management are often lacking, particularly in relation to long-term responses over decades of forest management. One of the best sources of information on long-term growth dynamics in response to management comes from long-term growth and yield plots with associated controls (Allen *et al.*, 1990; Stegemoeller & Chappell, 1990; Brix, 1993; Balster & Marshall, 2000; Jokela *et al.*, 2004). However, with relatively few exceptions, data collected in these long-term trials are focused on diameter and volume growth with other parameters only being measured infrequently for specific shorter-term studies. Very few of these trials have any long-term physiological data, and even those data span less than

a decade (Brix, 1993). Therefore, better understanding of the underlying physiological mechanisms behind growth increases is critical for predicting how forest carbon gain might respond to management under changing climate conditions.

Some past physiological responses to forest management can be obtained from these long-term experiments by analyzing the stable isotopes in tree-ring cellulose which record physiological and environmental processes at the time the ring was formed (McCarroll & Loader, 2004; Barbour, 2007). The carbon isotope ratio of plant tissue ($\delta^{13}\text{C}_{\text{plant}}$) reflects gas-exchange processes by the plant at the time the carbon was fixed, and is often used as an index of intrinsic water-use efficiency defined as the ratio of photosynthesis to stomatal conductance (A/g_s , Farquhar *et al.*, 1989b; see Materials and Methods section for details). The $\delta^{13}\text{C}$ in cellulose has been particularly useful for understanding responses to past management actions. For example, McDowell *et al.* (2003) noted that carbon isotope discrimination in tree-ring cellulose ($\Delta^{13}\text{C}_{\text{cell}}$) and growth increased

for dominant old-growth ponderosa pine trees after understory thinning. They concluded that an increase of water resource and g_s was responsible for the increased growth, and that this resource increase lasted over 15 yr. McDowell *et al.* (2006) also observed in ponderosa pine (*Pinus ponderosa*) that $\Delta^{13}\text{C}_{\text{cell}}$ decreased with increasing residual basal area of thinned stands from 5 to 12 yr after thinning. However, Martín-Benito *et al.* (2010) found no change in $\Delta^{13}\text{C}_{\text{cell}}$ after thinning in European black pine (*Pinus nigra*) stand, while other studies noted an increase in $\Delta^{13}\text{C}_{\text{cell}}$ with increasing basal area (Warren *et al.*, 2001; Powers *et al.*, 2010). Brooks & Coulombe (2009) found that while tree growth increased for over 20 yr in a Douglas-fir fertilization trial in the Wind River Experimental Forest, $\Delta^{13}\text{C}_{\text{cell}}$ decreased by 1.5‰ for only 4 yr after fertilization regardless of the amount of fertilizer applied. They speculated that the decrease was the result of increased leaf nitrogen increasing photosynthesis, while the later growth increases were attributed to gains in tree leaf area. Other fertilization trials have not shown such a decrease in $\Delta^{13}\text{C}_{\text{cell}}$ (Balster *et al.*, 2009), and interpreted their growth increases as increases in leaf area, not leaf nitrogen content.

To understand long-term carbon uptake dynamics, it would be useful to easily separate the effects of photosynthesis and stomatal conductance within A/g_s as indicated by $\delta^{13}\text{C}$. Several studies have indicated how the oxygen isotope ratio of plant tissue ($\delta^{18}\text{O}_{\text{plant}}$) might be useful in separating the effects of A from g_s in $\delta^{13}\text{C}_{\text{plant}}$, because the $\delta^{18}\text{O}_{\text{plant}}$ values are only influenced by water cycle (including g_s) and not by A (Scheidegger *et al.*, 2000; Grams *et al.*, 2007). The $\delta^{18}\text{O}_{\text{plant}}$ is influenced by the isotopic composition of soil water and atmospheric water, and evaporative enrichment of leaf water (Roden *et al.*, 2000; Barbour, 2007). In many long-term forest management experiments, treated and control plots are collocated, so $\delta^{18}\text{O}$ of soil water and atmospheric water vapor should be similar. Thus, differences in $\delta^{18}\text{O}_{\text{plant}}$ between treatments should result from differences in the evaporative enrichment of leaf water, which is influenced by g_s , relative humidity (RH), and leaf temperature (T_{leaf} , assuming air temperature is the same between plots). Brooks & Coulombe (2009) used $\delta^{18}\text{O}_{\text{cell}}$ to interpret changes in g_s because other sources of potential change to $\delta^{18}\text{O}_{\text{cell}}$ were either the same between treatment plots (source water and vapor $\delta^{18}\text{O}$) or were ruled out (RH and T_{leaf} ; see Discussion section for more details). They found that latewood $\delta^{18}\text{O}_{\text{cell}}$ values increased with fertilization and the duration of the increase above controls was longer for higher amounts of fertilization. They speculated that the increase of tree leaf area outpaced the increase in roots to support the leaf area resulting in stomatal closure during the late dry summers. Martín-Benito *et al.* (2010) observed that $\delta^{18}\text{O}_{\text{cell}}$ was higher after thinning, and suggested that the increase was related to a hotter, drier environment after thinning, and was not related to changes

in g_s since $\delta^{13}\text{C}_{\text{cell}}$ did not change with thinning. Thus, both thinning and fertilization have caused increases in $\delta^{18}\text{O}_{\text{cell}}$, but the speculated reasons for these increases were quite different.

Most of these long-term forest management experiments where isotopic analysis has been performed have only examined one management practice, such as different amounts of thinning or fertilization, but not a combination of treatments. Examining a combination of treatments might be particularly useful for helping to interpret $\delta^{18}\text{O}_{\text{cell}}$, since both canopy environment and plant water relations can change $\delta^{18}\text{O}_{\text{cell}}$ values (Sternberg, 2009). In this study, we used the Shawnigan Lake fertilization and thinning experiment to further develop the dual isotope ($\delta^{13}\text{C}_{\text{cell}}$ and $\delta^{18}\text{O}_{\text{cell}}$) approach under a wider range of management, where thinning, fertilization and the combination treatments were available. Shawnigan Lake has the added advantage in that measurements of soil moisture, leaf nitrogen, and foliage efficiency were made during the first 7 yr of the experiment, as well as many other measures to help interpret the stable isotope results.

Materials and Methods

Study site

The Shawnigan Lake experimental site is located on south-eastern Vancouver Island, BC, Canada (48.6336 N, 123.7122 W), and ranges from 330 to 375 m in elevation. The forest is within the Coastal Douglas-fir biogeoclimate zone that receives an average of 1160 mm of precipitation yr^{-1} with only 125 mm from June to September, with a mean annual temperature of 8.9°C. The soil is well-drained, coarse loamy and silty glacial till with a thin (2 cm) organic layer, and is classified as Orthic Dystic Brunisol (Crown & Brett, 1975). The site quality is considered nutrient-poor and the height at age 50 yr was estimated to be 25 m (Brix, 1993). The site was planted with 2-yr-old Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) in 1948 at a density of 2200 trees ha^{-1} after a stand-replacing fire in 1942 (Brix, 1993). Natural regeneration increased stand densities to *c.* 4500 trees ha^{-1} when the study was initiated. Tree canopies were closed with a gap > 2 m between understory and overstory foliage. The understory is dominated by salal (*Gaultheria shallon* Pursh), Oregon grape (*Mahonia nervosa* Nutt.) and bracken fern (*Pteridium aquilinum* (L.) Kuhn).

Study design

In 1971, thinning and fertilization plots were established in a complete randomized design when the trees were *c.* 24 yr old (Crown & Brett, 1975). In this study, we elected to include four of the nine treatments used at the site, since

these four had the greatest amount of historical data (Brix, 1983, 1993; Brix & Mitchell, 1983, 1986; Mitchell *et al.*, 1996). The selected treatments were as follows: control (T0F0); heavy thinning where two-thirds of the basal area was removed resulting in even spacing between residual trees (T2F0); fertilization with 448 kg N ha⁻¹ using urea (T0F2); and a combination of the thinning and fertilization treatments (T2F2). Each treatment was replicated twice on 0.08 ha plots with a 10.8 m treated buffer around each plot. Each plot has a 0.04 ha center for nondestructive measures and the outer plot for biomass harvesting. In 1988, 10 trees were harvested from each plot for biomass measurements (Mitchell *et al.*, 1996). Disks from these trees were collected at 1.5 m height, dried and stored. In 2008, three representative trees (disks) from that subsample were selected for isotopic analysis from each treatment plot for a total of six trees per treatment (24 trees total). Mean disk diameter was 20 cm (\pm 4.36 SD) and ranged from 12.6 to 28.8 cm. A 16 yr period (1966–1981) was selected for isotopic analysis that included 5 yr before treatment and 11 yr after treatments were applied.

Tree-ring and isotopic analysis

On each tree disk, ring width was measured along four pathways at 90° angles from each other from the pith to the disk edge, then averaged for the disk. All disks were aged and cross-dated using marker rings to ensure accurate dating (1970, 1971, and 1972 proved to be highly reliable marker rings for treated trees). Ring width was measured using tree-ring analysis systems (WinDENDRO, Reg 2006a; Regent Instruments Inc. Quebec, Canada) attached to a digital scanner (Epson Expression, 10000 XL supplied and calibrated by Regent Instruments). Disks were scanned at 2400 dpi and measured for latewood and earlywood boundaries to 0.001 mm accuracy. Each tree-ring image was visually adjusted for accurate boundary detection and then independently verified. Basal area increment (BAI) for latewood, earlywood and the entire ring was estimated using the average increment radius measurements from the four measured pathways and by assuming circular geometry to calculate area, and subtracting the area estimated from the previous increment radius.

Once disks were accurately aged and measured, three 10-mm-wide segments at 120° from each other were marked so that each year was uniquely identified, and then cut from the disk. The three segments were then cut into early- and latewood sections for each target year and combined into one late- and earlywood sample per yr, per tree (24 trees, 16 yrs, early- and latewood sections, giving 768 samples). Samples were ground to a fine powder using a ball mill (Spex 5300, Metuchen, NJ, USA), and extracted to yield α -cellulose (Sternberg, 1989; Leavitt & Danzer, 1993) and were analyzed for $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$.

Isotopes were measured on 1–2 mg subsamples that were either combusted in an elemental analyzer (ECS 4010; Costech, Valencia, CA, USA) for $\delta^{13}\text{C}$, or pyrolyzed in a high-temperature conversion elemental analyzer (TC/EA ThermoQuest Finnigan, Bremen, Germany) for $\delta^{18}\text{O}$ and 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 Environmental Protection Agency (EPA), Corvallis, OR, USA. All $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values are expressed relative to their respective standard Pee Dee belemnite (PDB) and Vienna Standard Mean Ocean Water (V-SMOW) in ‰:

$$\delta^{13}\text{C} \text{ or } \delta^{18}\text{O} = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) 1000, \quad \text{Eqn 1}$$

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

Modeling and statistics

To remove variation of $\delta^{13}\text{C}_{\text{air}}$ in $\delta^{13}\text{C}_{\text{cell}}$ values over time, $\delta^{13}\text{C}$ values were converted to $\Delta^{13}\text{C}$ using the following equation (Farquhar *et al.*, 1982):

$$\Delta^{13}\text{C} = \frac{\delta^{13}\text{C}_{\text{air}} - \delta^{13}\text{C}_{\text{cell}}}{1 + \delta^{13}\text{C}_{\text{cell}}/1000} \quad \text{Eqn 2}$$

$\delta^{13}\text{C}_{\text{air}}$ values were obtained from McCarroll & Loader (2004). Plant $\Delta^{13}\text{C}$ values are then used to estimate intrinsic water-use efficiency (A/g_s) using the following two equations (Farquhar *et al.*, 1989a):

$$\Delta^{13}\text{C} = a + (b - a) \left(\frac{c_i}{c_a} \right), \quad \text{Eqn 3}$$

where a is fractionation resulting from diffusion (4.4‰), b is fractionation associated with carboxylation by Rubisco (c , 27‰), and c_i/c_a is the ratio of internal [CO_2] to atmospheric [CO_2]. Note that $\Delta^{13}\text{C}$ should be directly related to the [CO_2] in the chloroplast (c_c) rather than c_i . As a result, using c_i may create complications if mesophyll conductance to CO_2 is limiting to A and not constant (Seibt *et al.*, 2008). Intrinsic water-use efficiency is estimated from c_i and c_a as follows:

$$\frac{A}{g_s} = \frac{(c_a - c_i)}{1.6}, \quad \text{Eqn 4}$$

where 1.6 is the ratio of diffusivities of water and CO_2 in air. A/g_s values estimated here are strongly dependent on the

parameter assumptions of the model and that mesophyll conductance does not limit A .

Treatment changes in $\delta^{18}\text{O}_{\text{cell}}$ were related to three possible driving factors: RH, T_{leaf} and g_s . The Craig–Gordon model describes the theoretical relationship between the variation in water $\delta^{18}\text{O}$ and RH (Craig & Gordon, 1965; Farquhar & Lloyd, 1993). This model describes water enrichment under steady-state conditions at the site of evaporation:

$$\Delta^{18}\text{O}_e = \varepsilon^* + \varepsilon_k + (\Delta^{18}\text{O}_v - \varepsilon_k) \frac{e_a}{e_t} \quad \text{Eqn 5}$$

where $\Delta^{18}\text{O}_e$ and $\Delta^{18}\text{O}_v$ represent the isotopic difference between source water and either leaf water at the site of evaporation or atmospheric water vapor, respectively. e_a/e_t is the ratio of ambient vapor pressure to saturated vapor pressure within the leaf (affected by leaf temperature). ε^* is the equilibrium fractionation factor for exchange between water liquid and vapor. ε_k is the kinetic fractionation that occurs during diffusion and can be calculated using stomatal and boundary layer conductances (g_b) to water vapor (Farquhar *et al.*, 1998; Barbour, 2007). This model is also affected by differences in leaf and air temperature through temperature effects on saturation vapor pressure.

While stomatal conductance can affect variation in $\delta^{18}\text{O}_{\text{cell}}$ through ε_k , the larger effect comes from the transpiration rate (E) and the back diffusion of enriched water into the leaf where sugars are being formed as described by the Péclet effect (\wp) for leaf water enrichment ($\Delta^{18}\text{O}_l$) (Barbour, 2007):

$$\Delta^{18}\text{O}_l = \frac{\Delta^{18}\text{O}_e(1 - e^{-\wp})}{\wp} \quad \text{Eqn 6}$$

and

$$\wp = \frac{LE}{CD} \quad \text{Eqn 7}$$

where L is the effective path length, E is transpiration, C is the molar density of water, and D is the diffusivity of H_2^{18}O . Stomatal conductance can be incorporated into Eqn 7 by substituting E with g_s (VPD) where VPD is the vapor pressure deficit. Determining L has proved to be quite difficult, and while some studies report it to be consistent within a species (Kahmen *et al.*, 2009), other studies indicate it could be quite variable (Ferrio *et al.*, 2009). Therefore we also estimate $\Delta^{18}\text{O}_l$ from the Craig–Gordon model without the Péclet. Roden & Ehleringer (1999) and Roden *et al.* (2000) incorporate a fraction of unenriched source water in leaf water as follows:

$$\Delta^{18}\text{O}_l = \Delta^{18}\text{O}_e(1 - p_v) \quad \text{Eqn 8}$$

where p_v is the proportion of leaf water not enriched by evaporation, and they found a value of 0.1 to be the best fit

for their data. However, this model limits the effect of stomatal conductance on $\Delta^{18}\text{O}_l$ by eliminating E from the calculation.

The incorporation of $\Delta^{18}\text{O}_l$ into cellulose of plant tissue ($\Delta^{18}\text{O}_{\text{cell}}$) is described using the following equation (Barbour & Farquhar, 2000):

$$\Delta^{18}\text{O}_{\text{cell}} = \Delta^{18}\text{O}_l(1 - p_{\text{ex}}p_x) + \varepsilon_o \quad \text{Eqn 9}$$

where p_{ex} is the proportion of oxygen atoms that exchange with source water during cellulose formation, and p_x is the proportion of unenriched water (xylem water) at the site of cellulose formation, which for wood collected from the main trunk is equivalent to 1. ε_o is a fractionation factor of +27‰ associated with the water/carbonyl interactions (Sternberg, 1989; Yakir & DeNiro, 1990).

Using these models, we varied either RH, T_{leaf} or g_s while the other two variables remained constant to explore the range for each driving variable to model the observed treatment changes in $\delta^{18}\text{O}_{\text{cell}}$. Our goal was to aid in interpreting the results through a model sensitivity analysis, rather than predict the exact values of the driving variables. We did not know the source water $\delta^{18}\text{O}$ value over time, so we could not directly calculate $\Delta^{18}\text{O}$ from our raw $\delta^{18}\text{O}_{\text{cell}}$ data. However, we assumed that source water did not differ between treatments so that all treatment variation in $\delta^{18}\text{O}_{\text{cell}}$ was the result of variation in treatment $\Delta^{18}\text{O}_l$ compared with control values. Thus, oxygen isotope data are presented as $\delta^{18}\text{O}_{\text{cell}}$ rather than $\Delta^{18}\text{O}_{\text{cell}}$, except for the modeling exercise where modeled $\Delta^{18}\text{O}_{\text{cell}}$ values are normalized by subtracting control values. Climate variables were set for average summertime conditions determined from the Shawnigan Lake Climate Station (unless that was the variable being changed): 20°C for air and leaf temperature, and 60% RH. Boundary layer conductance was set to 2, and stomatal conductance was set to 0.14, typical for Douglas-fir under nonwater-limited conditions (Bond & Kavanagh, 1999). We assumed that $\Delta^{18}\text{O}_v$ (Eqn 4) was in equilibrium with source water as is typical in well-mixed conditions, and thus was equal to $-\varepsilon^*$ (Barbour, 2007). We set p_{ex} to 0.4 and L to 3.3 cm, which is between the midpoint of the range and the mean for conifers (Wang *et al.*, 1998), but we also modeled the results without using the Péclet effect.

For statistical analysis, BAI, $\Delta^{13}\text{C}_{\text{cell}}$ and $\delta^{18}\text{O}_{\text{cell}}$ measurements were normalized to account for pretreatment differences by subtracting each tree's pretreatment (1966–1970) mean value from the values for each year in the tree's time series. For some analyses, treatment differences were estimated by subtracting the normalized control values from the normalized treated values. Repeated measures ANOVA was used to determine years with significant differences between treatments, and a Holm–Sidak Multiple Comparisons test was used to test for differences between treatments and

controls. All statistics were performed using SigmaStat (V 3.5, Systat Software Inc. Chicago, IL, USA). Error bars throughout the manuscript represent the standard error of the mean unless otherwise noted. Monthly climate data were obtained from the National Climate Data and Information Archive, Environment Canada, using the Shawnigan Lake Climate Station (ID 1017230), and the Palmer Drought Severity Index (PDSI) from the National Climate Data Center, the National Oceanic and Atmospheric Administration (NOAA).

Results

In the 5 yr before treatment (1966–70), the Douglas-fir trees where the plots were established were adding, on average, 950 mm² in basal area yr⁻¹. In the first 4 yr following treatment, fertilization and thinning increased tree growth by 69 and 53%, respectively, over pretreatment means, adding *c.* 600 mm² yr⁻¹ more (Fig. 1). The combination treatment tripled the growth rate, with the average tree gaining *c.* 1400 mm² yr⁻¹ at its peak. After the one-time treatment in 1971, BAI values for the fertilized (T0F2) and thinned trees (T2F0) were significantly greater than control trees until 1977 and 1978, respectively (Table 1). BAIs remained greater in the combined treatment trees (T2F2) through 1988 (end of the observation period). Most of the gain was a result of increased earlywood production; however, both thinned treatments also had significantly greater latewood growth relative to the controls in 1972–75.

Carbon isotope discrimination ($\Delta^{13}\text{C}_{\text{cell}}$) ranged from 16 to 21.7‰, a range of almost 6‰ among trees, treatments and years (Fig. 2). Earlywood $\Delta^{13}\text{C}_{\text{cell}}$ values were more variable within a year than latewood values. The variation between years for latewood control $\Delta^{13}\text{C}_{\text{cell}}$ values was highly correlated with mean annual precipitation ($r = 0.85$, $P < 0.001$) as well as other climate indicators of moisture (RH, PDSI, VPD) but to a lesser degree. Earlywood $\delta^{13}\text{C}_{\text{cell}}$ values for controls were also correlated with mean annual precipitation ($r = 0.58$, $P = 0.02$) and other moisture indicators, but the correlations were much weaker than for latewood.

Thinning alone had little effect on $\Delta^{13}\text{C}_{\text{cell}}$. Thinning (T2F0) decreased $\Delta^{13}\text{C}_{\text{cell}}$ in 1972 for earlywood, and increased $\Delta^{13}\text{C}_{\text{cell}}$ in 1971 for latewood (Fig. 2). Other than those two observations, $\Delta^{13}\text{C}_{\text{cell}}$ values in thinned trees (T2F0) did not significantly differ from control values.

Values of $\Delta^{13}\text{C}_{\text{cell}}$ decreased with fertilization (T0F2 and T2F2) relative to control values consistently for 3 yr after treatment in earlywood, and 4 yr for latewood (Fig. 2). Latewood $\Delta^{13}\text{C}_{\text{cell}}$ values for both fertilized treatments were significantly lower than control $\Delta^{13}\text{C}_{\text{cell}}$ values in other years through 1988 but not consistently. The years with significant $\Delta^{13}\text{C}_{\text{cell}}$ differences tended to be years with low annual precipitation (< 1000 mm). While fertilization alone decreased discrimination by 1.5‰ in the first few years after treatment, the combined treatment decreased discrimination by over 2‰ in both late- and earlywood. These decreases in $\Delta^{13}\text{C}_{\text{cell}}$ translated to increases in intrinsic

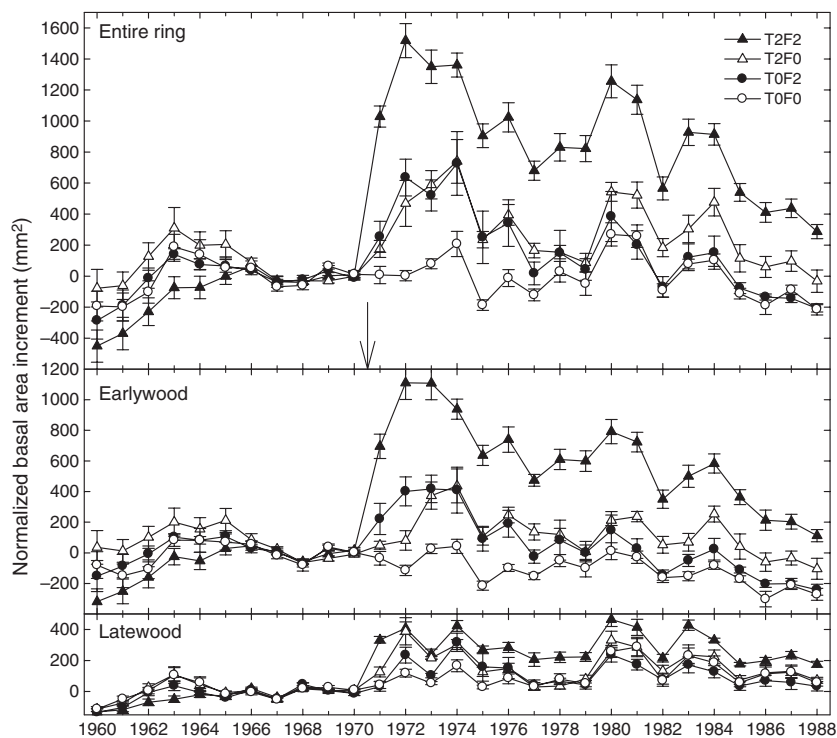


Fig. 1 Basal area increment (BAI) for trees ($n = 6$) in the four treatments: T0F0, control trees; T2F0, thinned trees; T0F2, fertilized trees; T2F2, combination. Values were normalized by subtracting the mean BAI for pretreatment years 1965–1970 for each tree. The arrow indicates the year that treatments were applied to the stand. Error bars are standard error of the mean. Statistical differences between treatments within a given year are shown in Table 1.

Table 1 Summary of significant statistical differences in basal area increment (Fig. 1) for the entire ring, latewood and earlywood (two-way repeated-measures ANOVA using a Holm–Sidak Multiple Comparisons test for differences between treatments and the control)

	Total basal area increment			Latewood basal area increment			Earlywood basal area increment		
Pretreatment years									
1960–1965	ns	ns	ns	ns	ns	ns	ns	ns	ns
1966–1970	ns	ns	ns	ns	ns	ns	ns	ns	ns
Treatment ^a	T0F2	T2F0	T2F2	T0F2	T2F0	T2F2	T0F2	T2F0	T2F2
1971	ns	ns	***	ns	ns	***	**	ns	***
1972	***	***	***	ns	***	***	***	*	***
1973	***	***	***	ns	**	**	***	***	***
1974	***	***	***	**	*	***	***	***	***
1975	***	***	***	**	ns	***	***	***	***
1976	**	**	***	ns	ns	***	**	***	***
1977	ns	*	***	ns	ns	**	ns	***	***
1978	ns	ns	***	ns	ns	**	ns	ns	***
1979	ns	ns	***	ns	ns	***	ns	ns	***
1980	ns	ns	***	ns	ns	***	ns	ns	***
1981	ns	ns	***	ns	ns	ns	ns	*	***
1982	ns	*	***	ns	ns	*	ns	**	***
1983	ns	ns	***	ns	ns	***	ns	*	***
1984	ns	**	***	ns	ns	**	ns	**	***
1985	ns	ns	***	ns	ns	***	ns	ns	***
1986	ns	*	***	ns	ns	ns	ns	*	***
1987	ns	ns	***	ns	ns	ns	ns	**	***
1988	ns	ns	***	ns	ns	***	ns	ns	***

Significant differences: *, $\alpha = 0.05$; **, $\alpha = 0.01$; ***, $\alpha = 0.001$; ns, not significant.

^aTreatment differences were determined by comparing to the 1966–1970 pretreatment period.

Treatments: T0F0, control; T2F0, heavy thinning where two-thirds of the basal area was removed resulting in even spacing between residual trees; T0F2, fertilization with 448 kg N ha⁻¹ using urea; T2F2, a combination of the thinning and fertilization treatments.

insic water-use efficiency (A/g_s) of 10–20 $\mu\text{mol mol}^{-1}$ (Fig. 3). The average A/g_s for controls was *c.* 70 $\mu\text{mol mol}^{-1}$, and thus the observed increase was *c.* 15–20% greater intrinsic water-use efficiency for the first 3–4 yr following fertilization.

To understand if these gains in A/g_s were related to increases in A , we compared these temporal increases with changes in leaf nitrogen and foliage efficiency (annual above-ground biomass production per unit foliage, kg kg⁻¹) previously reported by Brix (1983, 1993). Control trees had a leaf nitrogen content of *c.* 1%, and produced on average 1 kg of biomass for every kg of foliage annually, while thinned and fertilized trees (T2F2) reached a maximum of 2.65 kg kg⁻¹ (Brix, 1983) and nearly 2% leaf nitrogen content (Brix, 1993). The increases in both leaf nitrogen and foliage efficiency were also observed over the first 3–4 yr of treatments as they were for A/g_s . For earlywood, the treatment increases in A/g_s were linearly related to treatment increases in foliage efficiency (Fig. 4, $R^2_{\text{adj}} = 0.73$, $F = 63.3$). For latewood, only A/g_s for the fertilized treatments (T0F2, T2F2) are linearly related to foliage efficiency ($R^2_{\text{adj}} = 0.57$, $F = 20.9$), while A/g_s for thinned trees had no significant trend in foliage efficiency. The correlation with leaf nitrogen was much lower ($R^2_{\text{adj}} = 0.38$, $F = 10.8$), because leaf nitrogen peaked in

the year after fertilization and declined rapidly afterwards, whereas foliage efficiency and A/g_s peaked in the second year. Thinned trees had similar leaf nitrogen concentrations to controls over time (Brix, 1993).

Values for $\delta^{18}\text{O}_{\text{cell}}$ ranged from 26 to 33‰ over time and with treatment (Fig. 5). Pretreatment values were very consistent between trees within a year, but varied considerably between years for both early and latewood. Earlywood values were generally greater than latewood values within the same year. The annual variation in earlywood $\delta^{18}\text{O}_{\text{cell}}$ control values were correlated with climate variables, but the variation in latewood $\delta^{18}\text{O}_{\text{cell}}$ control values were not. Earlywood $\delta^{18}\text{O}_{\text{cell}}$ was highly correlated with spring (April–June) RH ($r = -0.57$, $P < 0.001$) and annual precipitation ($r = -0.52$, $P < 0.001$). However, latewood $\delta^{18}\text{O}_{\text{cell}}$ variation in controls was not correlated with any combination of seasonal, annual or monthly temperature, PDSI, RH, VPD or precipitation.

Values of $\delta^{18}\text{O}_{\text{cell}}$ showed significant responses to the treatments (Fig. 5). Once treatments were applied, $\delta^{18}\text{O}_{\text{cell}}$ values for the thinning treatments increased relative to control values, while fertilization $\delta^{18}\text{O}_{\text{cell}}$ values were similar to control values except for latewood in 1972, and earlywood in 1975. For earlywood, significant increases in $\delta^{18}\text{O}_{\text{cell}}$ as a result of thinning were not noted every year after treatments

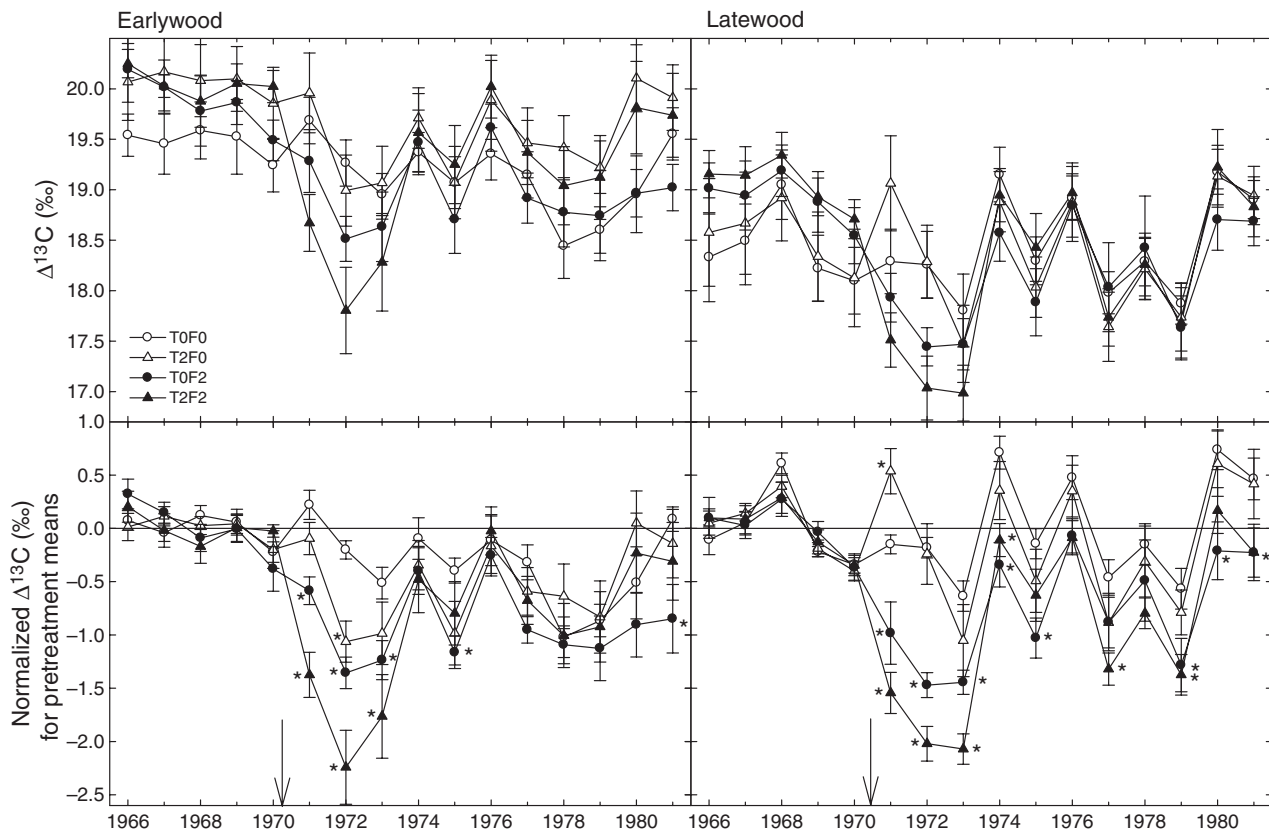


Fig. 2 Changes in carbon isotope discrimination ($\Delta^{13}\text{C}$) over time for each treatment. Treatments: T0F0, control trees; T2F0, thinned trees; T0F2, fertilized trees; T2F2, combination. The arrow indicates the time at which the treatments were applied to the stands. Asterisks (*) indicate significant differences ($\alpha = 0.05$) from control values in a given year. To normalize the data, the pretreatment means were subtracted from each tree's $\Delta^{13}\text{C}$ value in a particular year. Error bars are standard error of the mean.

were applied, but on alternate years (Fig. 6). These years tended to have drier spring seasons with low RH ($r = -0.59$, $P = 0.05$) and low PDSI scores, which indicated dry years ($r = -0.58$, $P = 0.055$). For latewood, the $\delta^{18}\text{O}_{\text{cell}}$ increases related to thinning were more consistent year to year, with significant differences noted for the first 3 yr. After that, significant increases in $\delta^{18}\text{O}_{\text{cell}}$ were only noted for the combined treatment and not thinning alone. The years with significant latewood differences did not have any particularly climate pattern. If anything, more humid climate was related to years with differences, but these were generally weak correlations.

Discussion

Trees responded dramatically to the thinning and fertilization treatments at Shawnigan Lake (Brix, 1983, 1993; Brix & Mitchell, 1983, 1986; Mitchell *et al.*, 1996), and stable isotope analysis of the tree-ring cellulose has helped to illuminate the different mechanisms for response. Decreases in $\Delta^{13}\text{C}_{\text{cell}}$ occurred in the fertilized trees and were short-lived, lasting only 3 or 4 yr for early- and latewood, respectively. These decreases indicate an increase in A/g_s and

were correlated with documented increases in leaf nitrogen and foliage efficiency (Brix, 1983, 1993). By contrast, increases in $\delta^{18}\text{O}_{\text{cell}}$ mostly occurred in the thinned trees, and the response over time was more variable. The $\delta^{18}\text{O}_{\text{cell}}$ values in thinned earlywood significantly increased over control values in drier spring seasons regardless of length of time since thinning occurred. Significant $\delta^{18}\text{O}_{\text{cell}}$ increases in latewood were not correlated with climate, but were more associated with the time since thinning, where most significant differences from control values occurred soon after thinning.

The sharp decrease in $\Delta^{13}\text{C}_{\text{cell}}$ and increase in A/g_s following fertilization was likely the result of increased A . Both foliage nitrogen concentrations and foliage efficiency are related to A (Brix, 1981, 1983; Field & Mooney, 1986) and were both correlated with the A/g_s dynamics observed in this experiment (Fig. 4). Foliage nitrogen increased from 1% to nearly 2% with fertilization in the first year after fertilization and then decreased rapidly such that N concentrations were similar to controls by year 4 (Brix, 1993). A/g_s was more closely related to foliage efficiency than to leaf nitrogen (Fig. 4), indicating that leaf nitrogen alone did not drive the changes in A related to fertilization.

Fig. 3 Changes in the ratio of photosynthesis to stomatal conductance (A/g_s) over time. Values were estimated from Eqns 2–4. Data were normalized for pretreatment means per tree, and then normalized control averages were subtracted from treatment averages for each year. Treatments: T2F0, thinned trees (open triangles); T0F2, fertilized trees (closed circles); T2F2, combination (closed triangles). The arrow indicates the year that treatments were applied to the stand. Asterisks (*) indicate significant differences ($\alpha = 0.05$) from control values in a given year. Error bars are standard error of the mean.

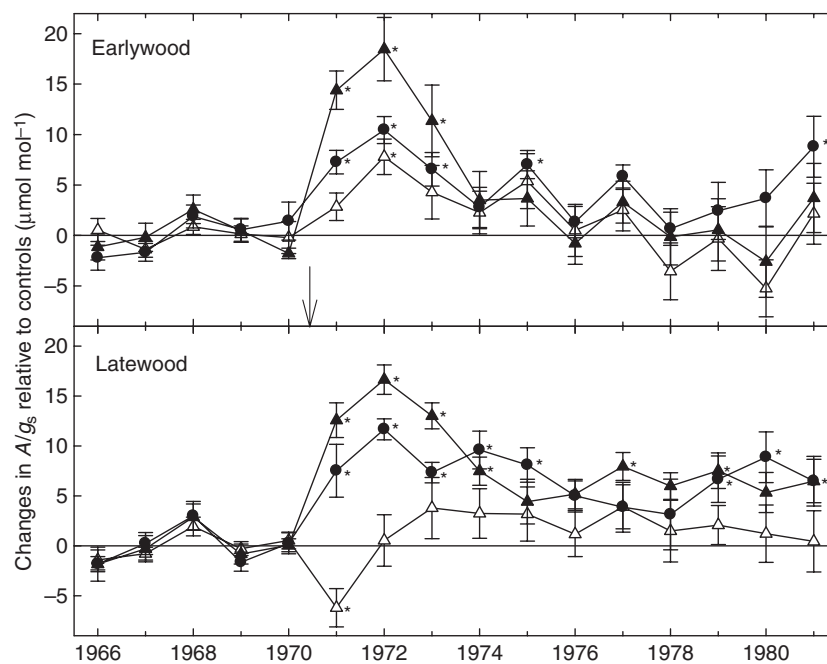
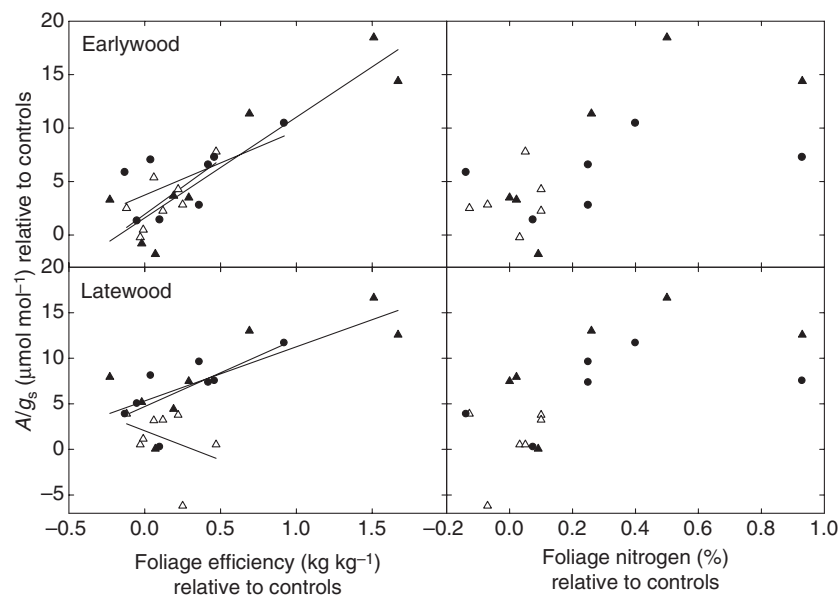


Fig. 4 The relationship between A/g_s (Fig. 3 data) and foliage efficiency (measured as kg of above-ground biomass produced per kg of foliage in a year, Brix, 1983) or foliage nitrogen concentrations (Brix, 1993; Mitchell *et al.*, 1996). All datasets were normalized by subtracting control means within a year from the treatment means. Lines are best-fit regression lines for each treatment. Treatments: T2F0, thinned trees (open triangles); T0F2, fertilized trees (closed circles); T2F2, combination (closed triangles).



Like A/g_s , foliage efficiency peaked in year 2, but returned to control values in year 4 (Brix, 1983). We ruled out any isotopic effects of source CO_2 from soil respiration since canopy foliage was at least 2 m from the ground (Buchmann *et al.*, 2002). Decreases in $\Delta^{13}\text{C}_{\text{cell}}$ could also be related to decreases in mesophyll conductance, not only increases in A/g_s (Flexas *et al.*, 2008; Seibt *et al.*, 2008). However, Mitchell & Hinckley (1993) noted that mesophyll conductance increased in fertilized Douglas-fir trees, which would increase $\Delta^{13}\text{C}_{\text{cell}}$, not decrease it as we observed. If mesophyll conductance also increased in the

fertilized Shawnigan Lake trees, then A/g_s values cited in Fig. 3 would be too low, since estimates using Eqn 3 assume that mesophyll conductance was constant between treatments. In addition, $\delta^{18}\text{O}_{\text{cell}}$ did not change with fertilization relative to the controls, indicating that g_s was likely similar to control values as well. Therefore, we conclude that A/g_s increased in fertilized trees because A increased from elevated foliar nitrogen and light exposure, while g_s did not change.

Brooks & Coulombe (2009) found similar short-term dynamics in $\Delta^{13}\text{C}_{\text{cell}}$ as a result of fertilization in Douglas-

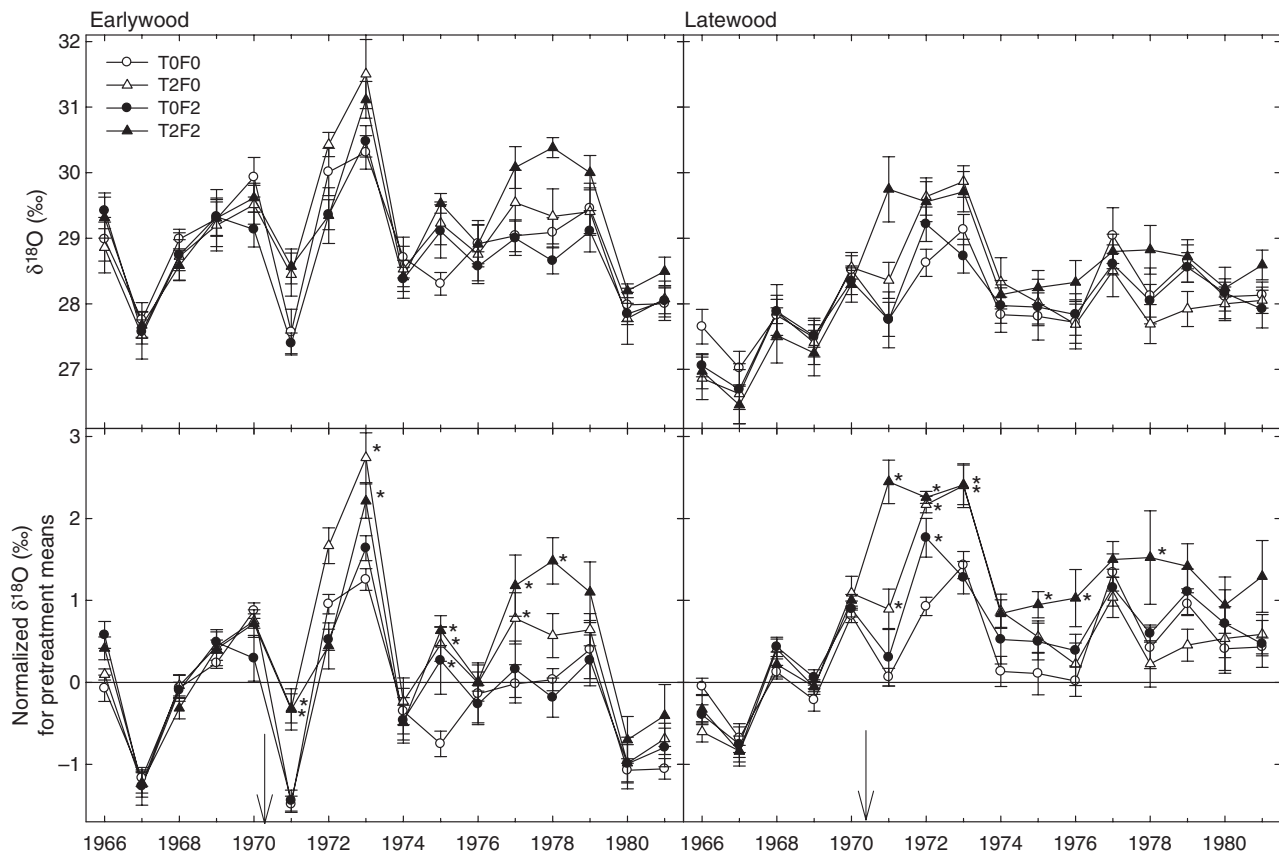


Fig. 5 Changes in oxygen isotope ratios ($\delta^{18}\text{O}$) over time for each treatment. TOF0, control trees; T2F0, thinned trees; TOF2, fertilized trees; T2F2, combination. The arrow indicates the time at which the treatments were applied to the stands. Asterisks (*) indicate significant differences ($\alpha = 0.05$) from control values in a given year. Error bars are standard error of the mean. To normalize the data, the pretreatment means were subtracted from each tree's $\delta^{18}\text{O}$ value in a particular year.

fir trees in Wind River, Washington. In that experiment, three different concentrations of nitrogen fertilization were used, and all three resulted in the same 1.5‰ decrease that was observed here in the fertilization-alone treatment. The highest N addition at Wind River was similar to the amount used in this experiment (471 kg N ha^{-1} at Wind River vs 448 kg N ha^{-1} at Shawnigan Lake). However, in this experiment the addition of thinning to fertilization had a larger effect on $\Delta^{13}\text{C}_{\text{cell}}$ with a drop of 2‰. These results indicate that the addition of nitrogen alone can only increase A/g_s by *c.* $10\text{--}15 \mu\text{mol mol}^{-1}$. Thinning would open the canopy, exposing more foliage to higher light intensities and causing an additional increase in A/g_s to $20 \mu\text{mol mol}^{-1}$. Thus the increase in A/g_s and foliage efficiency in the combined treatment was from higher leaf nitrogen and higher light intensities. Higher light intensities in the thinning-alone treatment did not increase A/g_s , but did slightly increase foliage efficiency. In fact, A/g_s decreased for latewood in the first year of treatment. We speculate that this may be a result of an increase in water resources from reduced competition with other trees. Brix & Mitchell (1986) noted that thinning increased soil

water potential during the dry summer period within this stand.

Not all fertilization experiments have resulted in a short-term increase in A/g_s (Balster *et al.*, 2009; J. R. Brooks, unpublished). Site fertility and rapid degree of growth response to fertilization likely influence leaf A/g_s during the first few years, and the rate of new foliage development. Both the Wind River and Shawnigan Lake experiments were on very low nutrient sites and had very dramatic responses to fertilization. Other fertilization trials have not shown such a dramatic response (Stegemoeller & Chappell, 1990; Hinckley *et al.*, 1992; Balster & Marshall, 2000; Jokela *et al.*, 2004). More isotopic retrospective analyses are needed of these long-term experiments to better understand leaf nutrient and leaf area interactions on $\Delta^{13}\text{C}_{\text{cell}}$.

In this experiment, $\Delta^{13}\text{C}_{\text{cell}}$ did not respond to thinning alone, except for an increase in discrimination for latewood in the first year of treatment, and a decrease in the second year for earlywood. Martín-Benito *et al.* (2010) also found no change in $\Delta^{13}\text{C}_{\text{cell}}$ in response to thinning of European black pine. However, ponderosa pine, both in the Pacific Northwest and in Arizona, increased $\Delta^{13}\text{C}_{\text{cell}}$ after thinning

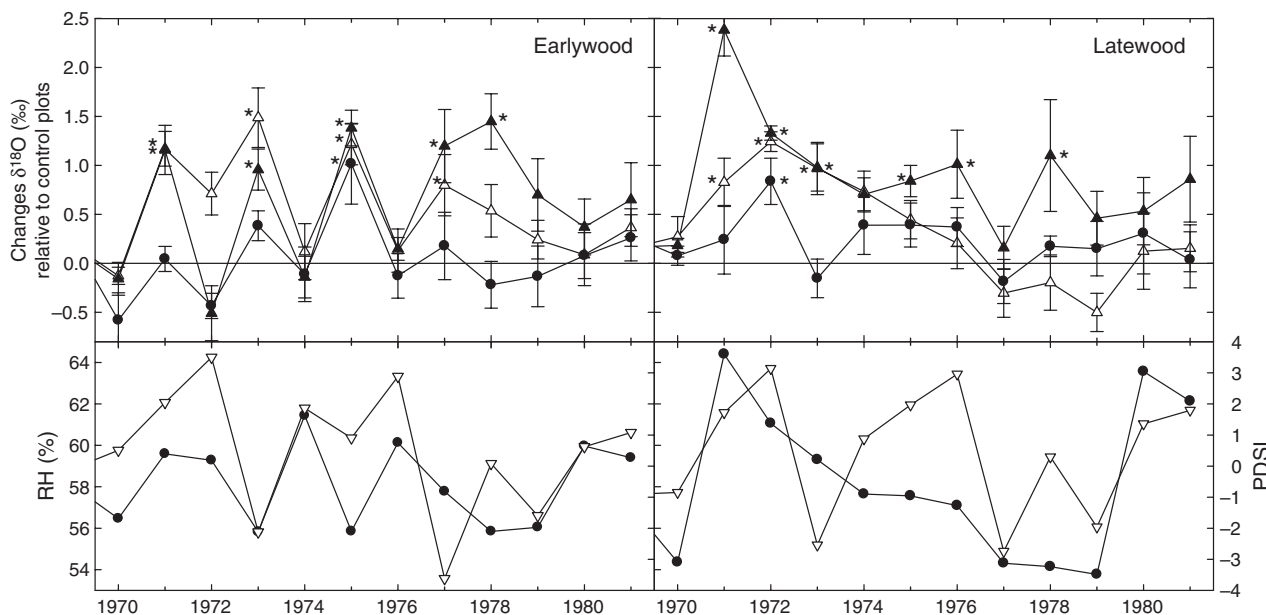


Fig. 6 Treatment changes in $\delta^{18}\text{O}$ relative to control plots and climate patterns over time. Earlywood and latewood: T2F0, thinned trees (open triangles); T0F2, fertilized trees (closed circles); T2F2, combination (closed triangles). Lower panels: relative humidity (RH) and Palmer Drought Severity Index (PDSI) for earlywood comparisons are the mean monthly averages for April, May and June. RH (closed circles) and PDSI (open triangles) for latewood comparisons are the mean June RH and the PDSI averages for July and August. These climate variables had the highest correlation with the changes in $\delta^{18}\text{O}$. Asterisks (*) indicate significant treatment differences from controls within a particular year. Error bars are standard error of the mean.

at least for some period of time (McDowell *et al.*, 2003, 2006). These differing results could be the result of two counteracting factors increasing with thinning: canopy light exposure and soil water supply. Increasing canopy light would increase A , while increasing soil moisture would increase g_s . In the ponderosa pine studies, the locations are drought-prone with relatively open canopies, and thus the increase in water supply after thinning was speculated to cause the increase in discrimination, while light intensities did not really change as a result of thinning. Warren *et al.* (2001) did note an increase in $\Delta^{13}\text{C}_{\text{cell}}$ with increasing pre-dawn water potentials in two species of pine, and this effect was greater at lower stand densities. As mentioned earlier for Shawnigan Lake, soil water potential was observed to increase in thinned stands (Brix & Mitchell, 1986), but light exposure also increased. Evidently this interaction between light and water supply caused A/g_s to remain stable in these thinned trees.

The increase in $\delta^{18}\text{O}_{\text{cell}}$ from thinning was likely the result of changes in canopy microclimate, namely decreases in RH and/or increases in leaf temperature (T_{leaf}) and their effect on e_a/e_i in Eqn 5, and not through a decrease in g_s and thus E in Eqn 7. If the mechanism was decreasing g_s , we would have expected to see a response in the fertilized trees where total stand leaf area was the highest, thus having the highest water depletion rates and being the most likely to close their stomata in late summer (Brooks & Coulombe, 2009). Since significant $\delta^{18}\text{O}_{\text{cell}}$ increases were

mostly found in the thinned trees where water resources increased relative to controls (Brix & Mitchell, 1986) and the $\delta^{18}\text{O}_{\text{cell}}$ increases occurred during dry springs (Fig. 6), changes in canopy microclimate between thinned and control stands seem most likely. Using Eqns 5–9, we estimated how much each of those three factors (RH, T_{leaf} and g_s) would have to change in order to obtain the treatment differences we observed in $\delta^{18}\text{O}_{\text{cell}}$ (Fig. 7). It is important to note that the degree of sensitivity for each variable to change $\delta^{18}\text{O}_{\text{cell}}$ is dependent on the initial value of all parameters. For example, using the Pécellet model, the $\delta^{18}\text{O}_{\text{cell}}$ response to g_s is much greater at lower RH, since E varies more in response to g_s when RH is low. In Fig. 7, we used average midsummer daytime values as our initial conditions, as these represent latewood conditions. Using the Pécellet model and keeping T_{leaf} and g_s constant, RH in the thinned stands had to decrease by 14% relative to the control stands to account for the observed $\delta^{18}\text{O}_{\text{cell}}$ range. However, increasing T_{leaf} as much as 6°C above control trees (same as air temperature = 20°C) could not increase the predicted $\Delta^{18}\text{O}_{\text{cell}}$ enough to account for the observed range. Further increases in T_{leaf} did not increase $\Delta^{18}\text{O}_{\text{cell}}$ relative to controls any more. Likewise, at an RH of 60% and a T_{leaf} of 20°C, decreasing g_s from 0.14 to 0 mol m⁻² s⁻¹ also could not account for the observed differences.

The model predicting leaf water enrichment using the Pécellet effect greatly dampens the effect of RH and T_{leaf} compared with earlier models which do not include the

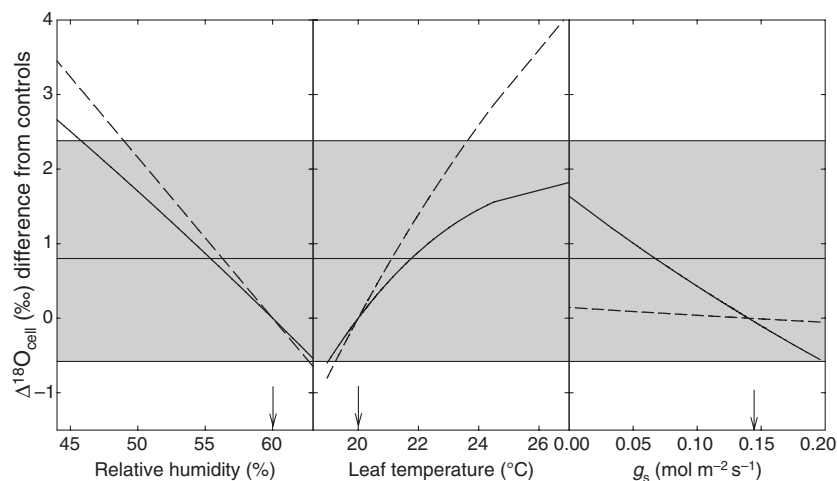


Fig. 7 Changes in controlling variables necessary in the models (Eqns 5–9) to cause the observed treatment differences in $\Delta^{18}\text{O}_{\text{cell}}$ from the control values. The arrows along the x-axis indicate values used in the models for the control treatments. Modeled responses of $\Delta^{18}\text{O}_{\text{cell}}$ are shown with the Pécelt effect (solid line, Eqns 6, 7) and without it (dashed line, Eqn 8). The gray area represents the range of $\delta^{18}\text{O}_{\text{cell}}$ differences from controls observed in this experiment. Observations above the horizontal line at 0.8‰ were significantly greater than control values.

Pécelt effect (Roden *et al.*, 2000). In addition, the Pécelt equation (Eqn 7) requires estimates of effective path length (L), which is difficult to determine and might or might not be constant during an experiment such as this (Ferrio *et al.*, 2009; Kahmen *et al.*, 2009). Using Eqn 8 instead of the Pécelt effect (Eqns 6, 7) decreased the amount by which RH and T_{leaf} would need to change in order to account for the observed treatment changes in $\delta^{18}\text{O}_{\text{cell}}$ (Fig. 7, dashed lines). However, this model excludes E , and thus limits the effects of g_s on $\delta^{18}\text{O}_{\text{cell}}$ to ε_k in Eqn 5. Using this model, RH would have to decrease from 60% in the control stands to as much as 49% in the thinned stands, and T_{leaf} would have to be a maximum of 3.7°C above leaf temperature in the control tree to account for these treatment changes in $\delta^{18}\text{O}_{\text{cell}}$. These microclimate differences seem more realistic. More likely, both of these variables changed simultaneously, which would decrease the necessary range for each variable even more. Decreases in RH and increases in temperature as a result of thinning have been noted in other studies within the range found here (Riegel *et al.*, 1992; Ma *et al.*, 2010). Therefore, we conclude that changes in canopy microclimate were responsible for the changes in $\delta^{18}\text{O}_{\text{cell}}$ as a result of canopy thinning.

Stomatal conductance could not account for the full range of $\delta^{18}\text{O}_{\text{cell}}$ variation using either model at this RH. Also, the lack of $\delta^{18}\text{O}_{\text{cell}}$ response to fertilization here differed from that found at Wind River, where fertilization dramatically increased latewood $\delta^{18}\text{O}_{\text{cell}}$, and the authors related the increase to g_s . In the Wind River fertilization experiment, Brooks & Coulombe (2009) estimated that g_s in fertilized trees decreased by as much as 50% in late summer to cause the range of variation they observed. One important difference between these experiments is that aver-

age midsummer RH was much higher at Shawnigan Lake (60% vs 33% at Wind River) because of the closer proximity of the ocean. A higher RH not only reduces the sensitivity of $\delta^{18}\text{O}_{\text{cell}}$ to g_s , but would also reduce total E for the site, making it much less likely for the fertilized trees with greatest stand leaf area to deplete soil water and close stomata relative to the controls. In the Wind River experiment, Brooks & Coulombe (2009) ruled out RH and T_{leaf} as possible drivers for the $\delta^{18}\text{O}_{\text{cell}}$ changes because fertilization increased leaf area, and thus increased canopy shading and the canopy boundary layer, decoupling the canopy from the ambient condition. If anything, these structural changes would increase RH within the canopy, and likely decrease T_{leaf} through shading, which would decrease $\delta^{18}\text{O}_{\text{cell}}$ rather than increase it. Decreases in g_s might cause increases in T_{leaf} sufficient to increase $\delta^{18}\text{O}_{\text{cell}}$, but latent heat exchange effects on T_{leaf} are not included in current $\delta^{18}\text{O}$ models.

In conclusion, we successfully used stable isotopes to examine the physiological mechanisms driving the growth responses to fertilization and thinning in the Shawnigan Lake thinning and fertilization experiment. $\delta^{13}\text{C}_{\text{cell}}$ was the most reliable indicator of physiological processes, while $\delta^{18}\text{O}_{\text{cell}}$ was largely responding to microclimate differences between stands. These isotope results concurred with the previous studies on the physiological mechanisms behind fertilization and thinning growth responses at Shawnigan Lake (Brix, 1993). This study continues to demonstrate that stable isotopes contained within tree rings can be used for retrospective analysis of physiological responses to management spanning decades, particularly if a nearby stand not subjected to management treatments can act as a control for separating climate effects. Future studies should obtain

these long-term isotopic records from a range of forests with different site and climate conditions in order to understand how the basic physiology behind forest carbon gain changes with management over timescales of decades.

Acknowledgements

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