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Basal area growth, carbon isotope discrimination, and intrinsic water use efficiency after fertilization of Douglas-fir in the Oregon Coast Range

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ABSTRACT

Many hectares of intensively managed Douglas-fir (*Pseudotsuga menziesii* Mirb. Franco) stands in western North America are fertilized with nitrogen (N) to increase growth rates, but only about ⅔ of all stands respond. Understanding the mechanisms of response facilitates prioritization of stands for treatment. The primary objective of this study was to test the hypothesis that the short-term basal area growth response to a single application of 224 kg N ha^{-1} as urea was associated with reduced stable carbon isotope discrimination ($\Delta^{13}C$) and increased intrinsic water use efficiency (*i*WUE) in a 20-yr-old plantation of Douglas-fir in the Oregon Coast Range, USA. Increment cores were measured to estimate earlywood, latewood, and total basal area increment over a time series from 1997 to 2015. Stable carbon isotope discrimination and *i*WUE were estimated using earlywood and latewood stable carbon isotope concentrations in tree-ring holocellulose starting seven years before fertilization in early 2009 and ending seven years after treatment. A highly significant ($p < 0.01$) interaction effect between fertilization treatment and year was found for total basal area growth and earlywood basal area increment. Specifically, fertilized trees showed significant responses ($p < 0.05$) in total basal area growth and earlywood basal area increment in the first (2009) and second (2010) growing seasons after fertilization in 2009. A marginally significant ($p < 0.10$) fertilization effect was found for latewood basal area increment only in the first growing season after treatment. A significant treatment × year interaction was also found for $\Delta^{13}C$ and *i*WUE in earlywood and latewood. Fertilization significantly reduced earlywood $\Delta^{13}C$ and increased earlywood *i*WUE in the first and second growing seasons after fertilization. Only a marginally significant fertilization effect was detected for latewood $\Delta^{13}C$ and *i*WUE in the second growing season after treatment. Previous studies of N fertilization of Douglas-fir forests have reported consistently increased growth and *i*WUE on low productivity sites treated with relatively high fertilization rates. This study suggested that these responses can also be observed on highly productive sites despite their lower frequency and apparently shorter duration. Other key mechanisms driving growth responses appear less important than *i*WUE, including an increase in LAI and shift from belowground to aboveground carbon allocation.

1. Introduction

Nitrogen (N) is the most important limiting nutrient in terrestrial ecosystems in most of the world (Field and Mooney, 1986; LeBauer and Treseder, 2008), including Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) ecosystems of the U.S. Pacific Northwest (PNW) and southwestern British Columbia (Miller et al., 1986; Gessel et al., 1990; Brix, 1991; Chappell et al., 1991; Hanley et al., 2006; Perakis and Sinkhorn, 2011). Nitrogen can limit plant growth on a given site for several reasons: (1) small N pools; (2) large N pools but low availability; (3) rapid leaching of available N below the root zone (particularly nitrate, NO_3^-); and (4) conversion of nitrate to gas by denitrifying bacteria (Field and Mooney, 1986; Binkley and Fisher, 2013). The importance of N to optimal

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physiological functioning of plants is directly related to its role as a fundamental component of amino acids, proteins, nucleic acids, and enzymes (including Rubisco), with particularly high concentrations in foliage as part of the photosynthetic apparatus (Field and Mooney, 1986; Binkley and Fisher, 2013).

Due to this key role of N, a positive relationship between photosynthetic rate and leaf N content has been reported for a wide variety of plant species including Douglas-fir (Brix, 1981; Field and Mooney, 1986; Mitchell and Hinckley, 1993; Ripullone et al., 2003; Manter et al., 2005; Duursma and Marshall, 2006). This relationship, however, requires sufficiently high intensity of incident photosynthetically active radiation (PAR; Brix, 1971; Tarvainen et al., 2016). The limitation that N availability places on forest productivity has been demonstrated repeatedly by N fertilization experiments. In one Douglas-fir field trial at Shawnigan Lake, British Columbia, leaf N concentrations of 1.74% shortly after fertilization increased photosynthetic rates by approximately 30% over that measured at leaf N concentrations of 1.0% in unfertilized plots (Brix, 1981). Although foliage N concentrations returned to pre-fertilization levels within 3–5 years after fertilization, a concurrent but slower increase in total leaf area (and total canopy N) contributed to longer-term growth responses relative to unfertilized control plots (Brix, 1981). Concurrent shifts in carbon allocation from belowground to aboveground tree components have also been required to explain the full magnitude of stem growth responses to fertilization (Gower et al., 1994; Lim et al., 2015). The aggregate response to N fertilization is therefore commonly conceived of as an initial increase in photosynthetic rate and growth efficiency (biomass or stem volume growth per unit of initial foliage), grading into shifts in carbon allocation and a longer term phase of elevated leaf area index (Brix, 1983; Lim et al., 2015).

Experimental research on operational N fertilization of Douglas-fir in the PNW began in the early 1950s in mid-rotation natural stands, generally with additions of ammonium nitrate, urea, or ammonium sulfate, and sometimes in combination with other elements such as P, K, and Ca in the form of lime (Gessel and Walker, 1956; Gessel and Shareeff, 1957). Observed growth responses to N fertilization at Shawnigan Lake and on larger plot networks in the Douglas-fir region have led to widespread operational N applications in Douglas-fir (Peterson and Hazard, 1990), amounting to 50,000–55,000 ha treated annually in the late 1980s through the 1990s (Chappell et al., 1991).

Despite this widespread N application and favorable average growth response in the PNW, it has long been recognized that approximately one third of fertilized Douglas-fir stands do not respond to N fertilization. In the current operational environment, it has become essential to optimize the economic and environmental performance of forest fertilization by applying N to only those stands or sites that can uptake the added N (preventing leaching and degradation of surface water) and accelerate growth (generating positive economic returns).

The Giustina fertilization trials in Douglas-fir were initiated to validate and refine the current conceptual model of mechanisms driving growth responses to N fertilization, with the ultimate goal of developing a site evaluation system derived from these mechanisms to provide improved predictions of site-specific growth responses, particularly on higher quality sites where responses are more erratic. Detailed physiological studies have long been needed, but are difficult to implement on a regional basis. However, analysis of carbon isotope ratios in annual growth rings before and after fertilization offer considerable promise for inferring physiological mechanisms driving growth responses to silvicultural treatments at a scale directly transferrable to operational prescriptions (Brooks and Coulombe, 2009; Brooks and Mitchell, 2011; Wei et al., 2014).

Stable isotopes of elements can serve as integrators or tracers of many key physical and biological processes (Sulzman, 2007; Dawson et al., 2002). Carbon fixation during the process of photosynthesis, for example, discriminates against the heavier stable isotope of carbon (^{13}C) in favor of the lighter isotope (^{12}C), but the intensity of this discrimination depends on environmental conditions such as vapor pressure deficit and soil-water availability, as they affect physiological responses like stomatal conductance. Therefore, the carbon stable isotopic composition of C_3 plant tissues is often expressed as carbon isotope discrimination ($\Delta^{13}C$), a parameter that has often been used to track how environmental conditions affect leaf gas exchange (Farquhar et al., 1982; Farquhar et al., 1989; Dawson et al., 2002; Marshall et al., 2007; Voelker et al., 2016). Carbon isotope discrimination is typically employed as a proxy for a plant's intrinsic water-use efficiency (*i*WUE), or ratio of net assimilation (A) to stomatal conductance (g_s).

Tree-rings are composed of annual increments of xylem tissue, so increment cores can be used to retrospectively estimate past diameter or basal area growth (Phipps and Whiton, 1988; Biondi and Qeadan, 2008; Voelker et al., 2008; Heres et al., 2012; Voelker et al., 2014). Furthermore, carbon stable isotopes in the tree rings record a canopy-integrated signal of annual leaf gas-exchange (Francey and Farquhar, 1982; Brooks and Coulombe, 2009; Gessler et al., 2014; Voelker et al., 2014). To the extent that growth responses to N fertilization are influenced by A/g_s, differences in carbon isotope discrimination should lend insight into the mechanisms of fertilization response. For example, Brooks and Coulombe (2009) measured tree-ring growth and both carbon and oxygen stable isotopes in response to three levels of N fertilization (157, 314, 417 kg ha^{-1}) in an 85 year-old Douglas-fir plantation located at Wind River Experimental Forest in Washington. The annual basal area increment (BAI) of these trees peaked in the third growing season after fertilization (1966), after which the values decreased slowly back to control-levels over the next 20 years. In response to N fertilization, $\Delta^{13}C$ was reduced and *i*WUE was increased in both earlywood and latewood components, but only for three years before returning to pretreatment levels. This short-term $\Delta^{13}C$ response was attributed to an increase in leaf N concentration and photosynthetic rate per unit leaf area, while longer-term growth responses were attributed to the increase in total leaf area observed in many other studies (Brix, 1983; Vose and Allen, 1988). Brooks and Mitchell (2011) found similar responses to N fertilization (448 kg N ha^{-1} as urea) in a 41 year-old Douglas-fir plantation located at Shawnigan Lake, Vancouver Island, BC. The direct effect of N on tree growth lasted six years after application, but $\Delta^{13}C$ was only reduced for the first few years, again related to an increase in leaf N and photosynthetic rate, prior to a subsequent increase in leaf area, which sustained the longer-term increase in growth. In contrast, Balster et al. (2009) did not find decreased $\Delta^{13}C$ in fertilized Douglas-fir plantations in the U. S. northern Rocky Mountains, despite a significant growth response to treatment. They speculated that their relatively coarse 3-yr growth period may have prevented detection of shorter-term response of $\Delta^{13}C$, and that increases in leaf area were largely responsible for the increases in growth, as demonstrated by previous analyses (Balster and Marshall, 2000).

Earlier studies of growth, $\Delta^{13}C$, and *i*WUE responses to N fertilization in Douglas-fir forests from the PNW region were conducted on lower productivity sites with treatments that exceeded standard industry practice for plantation management (Brooks and Coulombe, 2009; Brooks and Mitchell, 2011). The goal of the Giustina Fertilization Trials was to test the generality of apparent mechanisms of Douglas-fir response to N fertilization. Of particular interest was the degree of consistency in mechanisms at sites with much higher site index than either Shawnigan Lake or Wind River,

but with a demonstrated growth response to fertilization with 224 kg N ha^{-1} as urea. The project involved intensive felled-tree sampling from strips adjacent to the permanent plots for the first three years after fertilization, in addition to remeasurement of the central core plot. The felled tree data provided detailed description of crown structural responses to fertilization, including estimates of total leaf area (Mainwaring et al., in preparation). The primary objective of the analysis reported here was to test the hypothesis that fertilization reduced $\Delta^{13}C$ and increased *i*WUE, implying that the treatment had greater relative impact on *A* than g_s. A secondary objective was to provide perspective on the role of *i*WUE relative to other mechanisms driving stem growth responses in Douglas-fir. These other mechanisms included an increase in leaf area index (and implied PAR interception), shift in carbon allocation from belowground to aboveground components, and potential shift in carbon allocation from branch wood to stem wood.

2. Materials and methods

The Giustina trials were established in early 2009 under a generalized random block design in ten Douglas-fir stands in the Coast Ranges and western Cascades of Oregon. Selection criteria for subject stands included: total plantation age of 20 yrs (±5 years); tree density of 750 trees ha^{-1} (±250 trees ha^{-1}); and no thinning or fertilization within the previous seven years. Four 0.04-ha plots with 10-m buffers were established in each stand. Two of the four plots were randomly assigned to the unfertilized control treatment and two to the N fertilization treatment (224 kg N ha^{-1} as urea). Data from these trials were analyzed to test the null hypothesis that fertilization did not increase or decrease total stem volume growth per unit area. This null hypothesis was rejected ($p = 0.032$) based on the first three years of growth after the fertilization (2009–2011) (Mainwaring et al., 2013). The replication of each treatment at each site facilitated a test on the interaction between site and treatment, and this interaction was found marginally significant ($p = 0.092$). Previous regional fertilization trials similarly found wide variation among sites in response to fertilization, including many with no response (e.g., Peterson and Hazard, 1990). A multiple comparison test demonstrated that the fertilization effect on stem volume growth was significant at two of the ten sites. At the first of these two sites, Starker Forests (STT), the direct effect of fertilization was a 15.1% increase in volume increment over the control ($p = 0.0395$) and at the second, Plum Creek (PC), the direct effect of fertilization was a 22.7% increase ($p = 0.0044$). The 50-yr site indices (King, 1966) at STT and PC were 46.0 m and 45.5 m, respectively, indicating inherently productive sites. At the other eight installations site indices ranged from 41.2 m to 48.1 m, with an average of 45.7 m, so all installations occupied highly productive site class I land (King, 1966). Pre-treatment foliar N was weakly correlated with this relatively narrow range in site index ($r = 0.32$), ranging from 1.02 to 1.45% N by dry weight for all ten installations, and with an estimate of 1.23% for the control plots and 1.31% for the fertilized plots at STT.

The STT installation of the Giustina Fertilization Trials was selected as an intensive study site to test the effect of fertilization on $\Delta^{13}C$ and *i*WUE on a relatively moist and highly productive site. This replicate is located at 44°34′39″ North latitude and −123°29′35″ West longitude, at an elevation of 283 m. The soil is Ultic, with a loam texture (http://casoilresource.lawr.ucdavis.edu/, http://websoilsurvey.nrcs.usda.gov/app/HomePage.htm). Between 1997 and 2015 the average annual rainfall was 1677 mm, the January minimum temperature was 0.6 °C, the July maximum temperature was 26.7 °C, and the July maximum vapor pressure deficit averaged 2.2 kPa (http://www.prism.oregonstate.edu).

2.1. Tree measurements and wood sample collection

In November 2015, three trees were randomly selected from each of the four measurement plots at STT, including two control plots and two fertilized plots, yielding a total sample of 12 trees. On each sample tree, the diameter at breast height (DBH) was measured with a diameter tape (nearest 0.1 cm), and total height (THT) and height to crown base (TCHT) were measured with a hypsometer (nearest 0.01 m). Increment cores were collected from each tree using two sizes of increment borer. On each tree, a 5-mm diameter core was collected at breast height (1.3 m) on the uphill side of the tree, and three 12 mm diameter cores were collected at 90° intervals around the tree from the point at which the 5 mm sample was taken. The 5 mm cores were kept as reference cores for the future, while the 12 mm cores were used for isotopic analysis. The 36 12-mm increment cores were air dried, glued and mounted on wooden staves. The cores were sanded to a flat surface using 60 grit sandpaper mounted on a belt sander, and then with 220 grit sandpaper placed on an orbital sander. Breast height age was determined on the 12 mm cores after cross-dating.

2.2. Tree-ring measurements and basal area increment estimation

Tree-ring widths (total ring, earlywood, and latewood) were measured to the nearest 0.001 mm, using a Velmex sliding linear encoder device (Velmex, Inc., Bloomfield, NY, USA) and Measure J2X software (www.voortech.com/projectj2x/) on each 12-mm core. To visualize the tree ring image, a Fisher stereo microscope was used in companion with Micron digital imaging software. The tree-ring data were visually checked for accuracy on the total ring, latewood and earlywood widths. The cross-dating of tree-rings was checked using COFECHA software (Holmes, 1983; Grissino-Mayer, 2001). The tree-ring measurements covered the period from 1997 to 2015. Basal area increments (BAI) (mm^2 $year^{-1}$) over the same period were estimated assuming the cross-section of the tree conformed to a circle at the beginning and end of each annual growth period, and that diameter inside bark at breast height in 2015 (DIB_{15}) was the following function of DBH in 2015 (Hann, 2011):

$$DIB_{15} = 2.54 \cdot [0.92443655 \cdot \left(\frac{DOB_{15}}{2.54}\right)^{0.988866545} \cdot e^{[-0.034145503 \cdot (1-CR)^{0.5}]} \quad (1)$$

where DIB_{15} was estimated diameter inside bark at breast height (cm) in 2015 and DOB_{15} was DBH in the same year (nearest 0.1 cm). Increments in earlywood and latewood basal area for each year (EWBAI and LWBAI) likewise were computed assuming the stem cross-section at the beginning and start of each earlywood and latewood ring conformed to a circle.

2.3. Stable isotope analyses

After ring-width measurements were completed, a rotatory Dremel 400 XPR with a 60 grit sanding band was used to grind the rings into wood powder. The three 12 mm cores from each tree were pooled into one sample per ring for earlywood and latewood separately for a total of 336 samples (12 trees, 14 years (2002–2015), earlywood and latewood sections). Wood powder was placed into labeled plastic micro-centrifuge tubes. Wood powder was then placed in filter bags (ANKOM technology, NY) and extracted to holocellulose following methods modified after Leavitt and Danzer (1993).

Approximately 0.8–0.9 mg of holocellulose was weighed into small (5 x 8 mm) tin capsules for stable carbon isotope analyses. The samples were then flash combusted using a Carlo Erba (NA

1500, Milan, Italy) elemental analyzer connected in continuous-flow mode to a DeltaPlus (Thermo Finnigan, Bremen, Germany) stable isotope ratio mass spectrometer located at the Stable Isotope Laboratory in the College of Earth, Ocean and Atmospheric Sciences (CEOAS), Oregon State University (OSU). The runs were calibrated daily using the international and in-house standards (replicate analyses of an in-house standard yielded a standard deviation of 0.07‰). Two to three samples were repeated in every run and the standard deviation of those repeats for earlywood was 0.82‰ and for latewood was 1.04‰.

Values of $\Delta^{13}C$ were determined in parts per mil (‰) relative to the VPDB standard based on the following equation (Farquhar et al., 1982; McCarroll and Loader, 2004):

$$\delta^{13}C = \left(\frac{R_{sample}}{R_{standard}} - 1\right)1000 \quad (2)$$

where R_{sample} and $R_{standard}$ are the $^{13}C/^{12}C$ ratios in a sample and standard, respectively. $\Delta^{13}C$ was further converted to $\Delta^{13}C$ over the retrospective sampling period with the following equation (Farquhar et al., 1982):

$$\Delta^{13}C = \frac{\delta^{13}C_{air} - \delta^{13}C_{cell}}{1 + \delta^{13}C_{cell}/1000} \quad (3)$$

where $\Delta^{13}C_{air}$ is the isotopic concentration from the air in a given year recorded at Mauna Loa, Hawaii (http://cdiac.ornl.gov/) and $\Delta^{13}C_{cell}$ is the value of the α-cellulose formed in that year.

Following Farquhar et al. (1982, 1989) $\Delta^{13}C$ values were further used to estimate the ratio of the concentrations of CO_2 in the leaf intercellular spaces to that in the atmosphere (c_i/c_a) as:

$$\left(\frac{c_i}{c_a}\right) = (\Delta^{13}C - a)/(b - a) \quad (4)$$

where a is the fractionation of 4.4‰ due to diffusion through stomata and b is the fractionation of 27‰ occurring during carboxylation by the Rubisco enzyme in chloroplasts. Values of c_i/c_a were then used with growing season c_a from Mauna Loa to estimate intrinsic water use efficiency (*iWUE*) as:

$$iWUE = \frac{A}{g_s} = \frac{(c_a - c_i)}{1.6} \quad (5)$$

where A is assimilation, g_s is stomatal conductance and the constant 1.6 represents the ratio of stomatal conductance to water vapor to that for CO_2 (Osmond et al., 1980; Farquhar et al., 1989, p. 3–8 in Ehleringer et al., 1993; Seibt et al., 2008).

2.4. Statistical analysis

The responses $\Delta^{13}C$ and *i*WUE were standardized to account for pre-treatment differences (Brooks and Mitchell, 2011). For both $\Delta^{13}C$ and *i*WUE, the average of each individual tree over a seven year pre-treatment period from 2002 to 2008 was subtracted from its value in each individual year of the entire series from 2002 to 2015.

The full statistical model to test N fertilization effects was as follows:

$$y_{ijk} = \mu + \tau_i + \alpha_j + \tau \cdot \alpha_{ij} + \theta_{cov} + \delta + \gamma + \varepsilon_{ijk} \quad (6)$$

where y_{ijk} = total ring basal area increment (TOTBAI), earlywood basal area increment (EWBAI), latewood basal area increment (LWBAI), standardized $\Delta^{13}C$, or standardized *i*WUE in the i^{th} treatment in the j^{th} year on the k^{th} tree; μ = the overall mean; τ_i = the effect of the i^{th} treatment (fixed parameter); α_j = the effect of the j^{th} year (fixed parameter); $\tau{\cdot}\alpha_{ij}$ = the interaction effect between the i^{th} treatment and the j^{th} year (fixed parameter); θ_{cov} = initial (early 2009) plot basal area (m^2 ha^{-1}) at time of fertilization (tested as a covariate only for the growth variables); δ was a random plot effect within treatments; γ was a random tree effect within plots; and ε_{ijk} = a random error associated with k^{th} tree in the j^{th} year from the i^{th} treatment. Effects were considered "highly significant" if $p < 0.01$, "significant" if $p < 0.05$, and "marginally significant" if $p < 0.10$. Initial basal area was dropped as a covariate due to lack of even marginal statistical significance as a predictor for basal area increment, earlywood increment, or latewood increment ($p = 0.651$, 0.454, and 0.175, respectively).

To accommodate the repeated measurements within the individual trees PROC MIXED (SAS version 9.4, SAS Institute, Cary, North Carolina, USA) was used to fit the statistical model with alternative variance-covariance structures for successive BAIs within a tree (Littell et al., 1998, 2006). The AR (1) variance-covariance structure was selected due to its low values of AIC, AICC, and BIC relative to alternative variance-covariance models. Least square means of TOTBAI, EWBAI, LWBAI, $\Delta^{13}C$, and *i*WUE were computed for each treatment in each year and the slice option was used to compare the least squares means of the fertilization effect by F-test for each year (Littell et al., 2006).

2.5. Quantification of contributing mechanisms of stem growth response to fertilization

To address the secondary objective of providing perspective on treatment effects imposed through increases in *i*WUE relative to other driving mechanisms, the amounts attributable to three other possible mechanisms were explored. These other mechanisms included the increase in light interception (inferred from the increase in LAI), a potential shift in allocation from branch wood to stem wood, and a potential shift from belowground to aboveground production. LAI was estimated from a combination of felled tree sampling and remeasurement of the permanent core plots (Mainwaring et al., in preparation). Because an increase in LAI does not imply a proportional increase in intercepted photosynthetically active radiation (iPAR), Smith's (1993) empirical equation based on Beer-Lambert principles was applied to estimate the increase in iPAR from the LAI estimates. The equation was developed for Douglas-fir and includes a correction for stand density as measured by Curtis's (1982) relative density (RD). The plots in this study were almost contiguous, so incident PAR on the canopy was assumed equivalent. Scaled growth was first calculated as the ratio of growth to % iPAR on fertilized plots. This ratio was then multiplied by the increase in % iPAR inferred from Smith's (1993) modified Beer-Lambert equation to estimate the increase in NPP attributable to the increase in LAI and light interception. The increases in stem wood (wood + bark), branch wood, and foliage biomass were estimated after standardizing for slight differences in the initial (pre-treatment) amounts of each component among plots within the same treatment. The proportion and absolute amount of total aboveground NPP attributable to fertilization effects from each mechanism were then estimated from these measured responses.

3. Results

In general, the sizes and breast height ages of the twelve sampled trees at STT were similar in 2015 when cores were collected (Table 1). Significant treatment × year interactions were detected for TOTBAI ($p = 0.0005$), EWBAI ($p = 0.0003$), and LWBAI ($p = 0.0255$). Neither the plot (δ) nor tree (γ) random effects were significant. Nitrogen fertilization had a significant effect on TOTBAI during the first and second growing seasons after application ($p = 0.0101$ and 0.0156, respectively) (Fig. 1). Nitrogen fertilization also had a significant effect on EWBAI during the first and second

Table 1
Diameter at breast height (DBH), total height (THT), total crown height (TCHT), and breast height age of the six trees sampled in the unfertilized control and fertilized with 224 kg N ha^{-1} treatments in the Starker Forests replication (STT) of the Giustina Fertilization Trial.

	TREATMENT			
	Unfertilized Control		Fertilized with 224 kg N ha^{-1}	
VARIABLE	MEAN	± SE	MEAN	± SE
DBH (cm)	30.67	0.77	32.4	1.20
THT (m)	24.32	0.15	25.0	0.54
TCHT(m)	12.25	0.25	12.43	0.29
BH AGE (yrs)	23.6	0.30	23.78	0.33

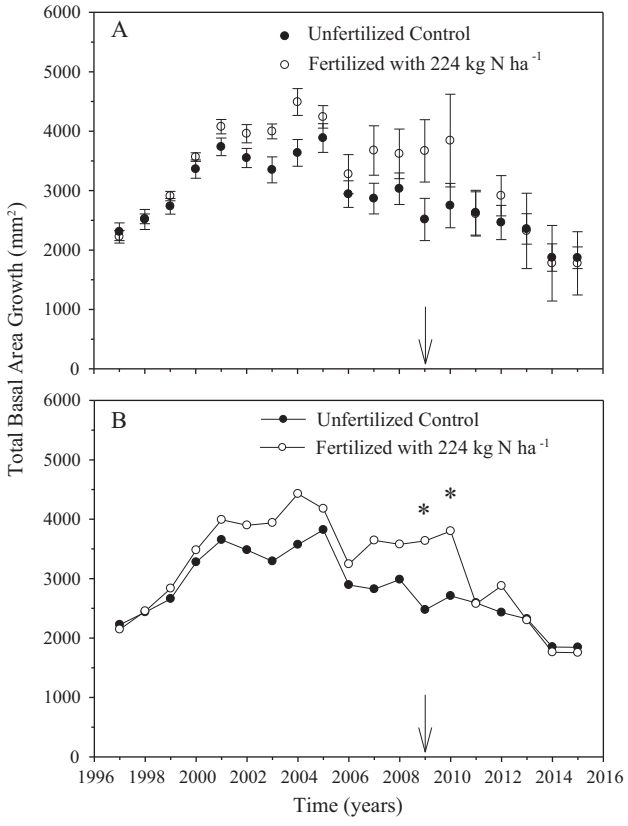

Fig. 1. (A) Mean total basal area increment (TOTBAI) with standard errors, and (B) least square means of TOTBAI for unfertilized control trees and trees fertilized with 224 kg N ha^{-1} in the STT replication of the Giustina Fertilization Trial (n = 6 trees per treatment). The arrow indicates when the fertilizer was applied. * = significant $\alpha = 0.05$.

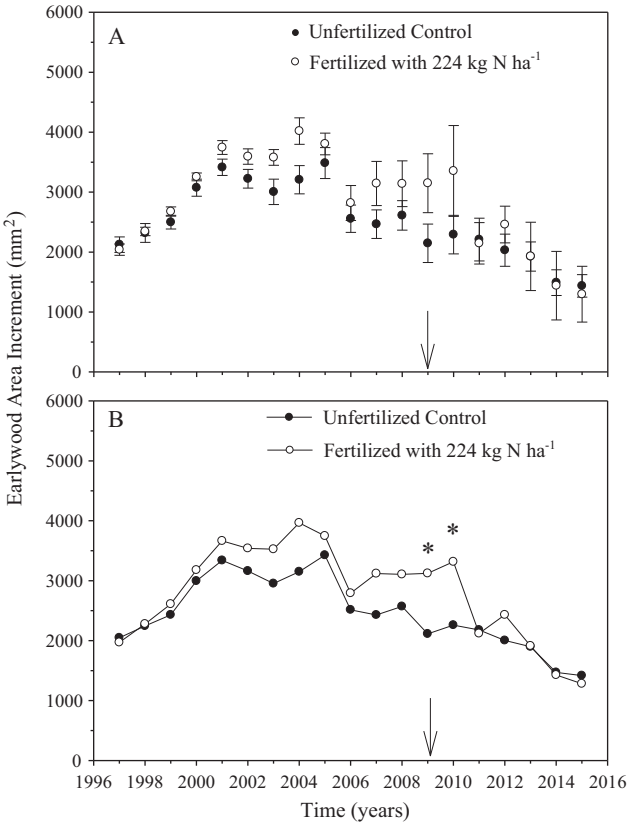

Fig. 2. (A) Mean earlywood area increment (EWBAI) with standard errors, and (B) least square means EWBAI for unfertilized control trees and trees fertilized with 224 kg N ha^{-1} in the STT replication of the Giustina Fertilization Trial (n = 6 trees per treatment). The arrow indicates when the fertilizer was applied. * = significant effect $\alpha = 0.05$.

growing seasons after application ($p = 0.0150$ and $p = 0.0109$, respectively) (Fig. 2). In contrast, N fertilization had a significant effect on LWBAI in only the first growing season after application ($p = 0.0466$; Fig. 3) (see Table 2).

Carbon isotope discrimination and *i*WUE displayed significant treatment × year interactions in both earlywood ($p = 0.0313$, $p = 0.0230$, respectively) and latewood ($p = 0.0230$, $p = 0.0223$, respectively) (Table 3). In the earlywood, N-fertilized trees had significantly lower $\Delta^{13}C$ compared to the control trees during the first and second growing season after treatment application ($p = 0.0011$; $p = 0.0170$, respectively) (Table 4). Likewise, in the earlywood, N-fertilized trees had significantly higher *i*WUE compared to the control trees during the first and second growing season after treatment application ($p = 0.0010$; $p = 0.0130$, respectively) (Fig. 4). In the latewood, N-fertilized trees had significantly lower $\Delta^{13}C$ ($p = 0.0159$) (Table 4) and higher *i*WUE ($p = 0.0154$) than the control trees only in the second growing season after treatment application (Fig. 5).

Earlywood $\Delta^{13}C$ for the seven years prior to fertilization was approximately 0.5‰ lower in the fertilized plots than in the control plots. This difference between treatments widened after fertilization for earlywood $\Delta^{13}C$ to 1.2‰ and 1.1‰ in 2009 and 2010, respectively, for a net fertilization effect of 0.7‰ and 0.6‰, respectively. This reduction in $\Delta^{13}C$ therefore corresponded to an increase of 7.0% and 6.2% in *i*WUE compared to the control trees. Latewood $\Delta^{13}C$ for the seven years prior to fertilization was approximately 0.1‰ lower in the fertilized plots than in the control plots. The post-fertilization difference in latewood $\Delta^{13}C$ of 0.7‰ in 2010 gave a net fertilization effect of 0.6‰. This reduction in $\Delta^{13}C$ corresponded to a 6.7% increase in *i*WUE during the 2nd growing season.

The proportion of sample tree basal area increment composed of earlywood and latewood for the first three years after

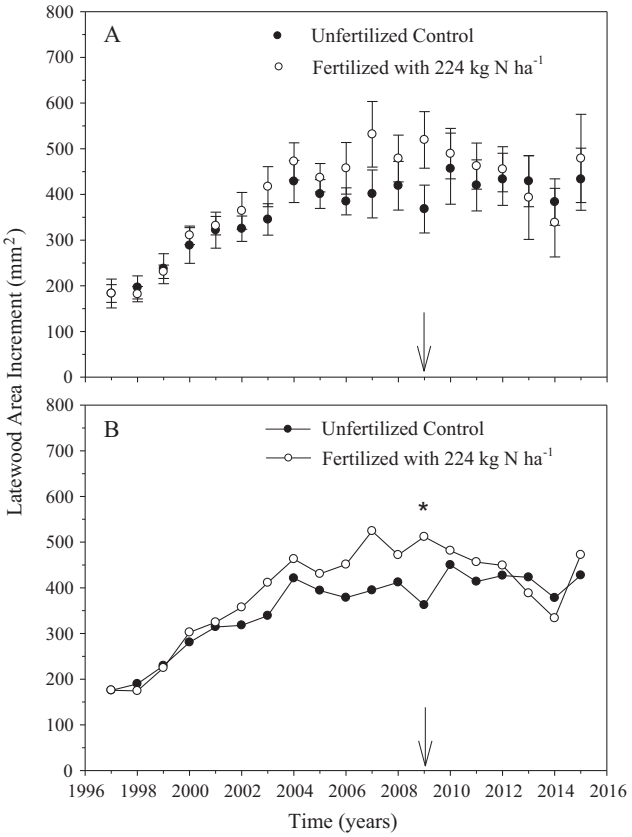

Fig. 3. (A) Mean latewood area increment (LWBAI) with standard errors, and (B) least square means of LWBAI for unfertilized control trees and trees fertilized with 224 kg N ha^{-1} in the STT replication of the Giustina Fertilization Trial (n = 6 trees per treatment). The arrow indicates when the fertilizer was applied. * = significant effect $\alpha = 0.05$.

fertilization was approximately 0.856 and 0.144, respectively, for fertilized trees, and 0.844 and 0.156 respectively, for control trees. Assuming that the contribution of earlywood and latewood to stem volume or biomass growth was proportional to their relative contribution to basal area increment for the first three years after fertilization, the weighted mean increase in *i*WUE attributable to fertilization would be approximately 5.44%.

The 3-yr increase in LAI on control plots was 1.94 $m^2 m^{-2}$ (8.16–10.10) and on fertilized plots it was 3.23 $m^2 m^{-2}$ (8.54–11.77). Standardizing to the slight difference in initial LAI between the control plots and fertilized plots, the fertilization effect on LAI was reduced slightly from 1.30 to 1.18, suggesting a 14.1% increase in LAI. However, the increase in LAI does not imply a proportional increase in intercepted photosynthetically active radiation (iPAR) due to light attenuation through the canopy. When Smith's (1993) modified Beer-Lambert equation was applied to LAI estimates for 1998 and 2011, fertilization was estimated to increase iPAR by only 1.47% over control plots. After accounting for the slight initial difference in pre-treatment LAI, the fertilization effect declined to a 1.26% increase in iPAR.

After standardization to a common initial aboveground biomass, fertilization was estimated to increase stem wood (wood + bark), branch wood, and foliage production by 1356.8, 394.6, and 331.1 kg $ha^{-1} yr^{-1}$, respectively, for a total of 2082.5 kg $ha^{-1} yr^{-1}$. These fertilization responses corresponded to a 3.7%, 7.0%, and 11.8% increase in stem wood, branch wood, and foliage mass over the control plots, and an increase of 5.6% in total ANPP.

4. Discussion

Analysis of stable C isotope ratios in wood formed during this fertilization trial led to two key results about response to fertilization. First, the immediate increase in stem wood growth was contemporaneous with the reduction in $\Delta^{13}C$ and increase in *i*WUE. Second, the increase in total aboveground productivity (ANPP) was approximately proportional to the increase in iWUE, averaging about 5.60% and 5.44%, respectively. Despite a 14% increase in LAI, the gain in light interception (iPAR) was very small, reaching only 1.26%. A shift from belowground to aboveground carbon allocation therefore was not necessary to account for the fertilization effect on stem growth or even total ANPP.

The immediate growth responses on this high quality site differed slightly from the lag in growth response observed in other trials on very low quality sites and receiving generally higher doses of N. Therefore, this difference is discussed briefly below from the viewpoint of providing potential insight into mechanisms of growth response that, perhaps with a bit of follow up work, could help address an operational issue of key current importance, i.e., the need for more accurate prediction of growth responses on relatively high quality sites, and resulting improvements in the economic and environmental performance of fertilized stands.

Growth responses to fertilization have historically been more variable on higher quality sites than on lower quality sites, but the potential gain in absolute volume growth is much greater on higher sites for a given percentage growth response. Considerable incentive therefore exists for identifying high quality sites that will respond, particularly if responses were a consequence of restoring levels of N availability that were diminished by harvest removals, site preparation, or other management practices. N applied as urea at operational levels for managed forests of western Oregon and Washington (224 kg N ha^{-1}) increased both growth and *i*WUE of the Douglas-fir growing on the STT site, a site of very high inherent productivity (site class I; King, 1966).

Growth responses were significant in both the earlywood and latewood at STT, but the magnitude was much higher early in the growing season when drought stress is typically minimal. Growth responses were contemporaneous with increases in *i*WUE, so were strongest in the first and second year after the fertilizer was applied. The immediate direct effects of fertilization on growth at STT in the growing season following fertilization contrasted with the one-year lag observed in low productivity stands at the

Table 2
Main treatment effect and interaction effect on total basal area increment (TOTBAI), earlywood area increment (EWBAI), and latewood area increment (LWBAI) in the Starker Forests replication (STT) of the Giustina Fertilization Trial.

	Variable					
	TOTBAI ($mm^2 year^{-1}$)		EWBAI ($mm^2 year^{-1}$)		LWBAI ($mm^2 year^{-1}$)	
Effect	F Value	Pr > F	F Value	Pr > F	F Value	Pr > F
Treatment	1.48	0.2510	1.47	0.2528	0.45	0.5189
Year	11.91	<0.0001	12.22	<0.0001	5.80	<0.0001
Treatment * Year	2.66	0.0005	2.79	0.0003	1.82	0.0255

Table 3
Main treatment effect and interaction effect on standardized carbon isotope discrimination ($\Delta^{13}C_{standard}$) and standardized intrinsic water use efficiency ($iWUE_{standard}$) of the earlywood and latewood in the Starker Forests replication (STT) of the Giustina Fertilization Trial.

	Earlywood				Latewood			
	$\Delta^{13}C$ standard (‰)		$iWUE_{standard}$ (μmol mol^{-1})		$\Delta^{13}C$ standard (‰)		$iWUE_{standard}$ (μmol mol^{-1})	
Effect	F Value	Pr > F	F Value	Pr > F	F Value	Pr > F	F Value	Pr > F
Treatment	2.76	0.1275	3.47	0.0922	0.81	0.3906	0.86	0.3743
Year	8.06	<0.0001	8.91	<0.0001	21.63	<0.0001	19.59	<0.0001
Treatment * Year	1.94	0.0313	2.03	0.0230	2.03	0.0230	2.04	0.0223

Table 4
Mean carbon isotope discrimination ($\Delta^{13}C$) of the earlywood and latewood in the unfertilized control and fertilized with 224 kg N ha^{-1} in 2009 treatments from 2002 to 2015 of the STT replication of the Giustina Fertilization Trial (n = 6). * = significant effect $\alpha = 0.05$; ** = highly significant effect $\alpha = 0.01$ as was determined by the F-test from PROC MIXED using the standardization method.

	$\Delta^{13}C$ (‰)			
	Earlywood Treatment		Latewood Treatment	
Year	Unfertilized control	Fertilized with 224 kg N ha^{-1}	Unfertilized control	Fertilized with 224 kg N ha^{-1}
2002	16.47	16.06	15.22	15.57
2003	16.23	16.08	15.24	15.60
2004	17.05	16.45	17.13	16.65
2005	17.30	16.65	16.47	16.34
2006	16.67	16.03	16.08	15.83
2007	16.61	16.23	16.72	16.30
2008	17.13	16.29	16.63	16.34
2009	17.38	16.19**	17.03	16.62
2010	17.37	16.26*	17.59	16.84*
2011	17.37	16.55	16.96	16.84
2012	17.01	16.46	16.40	16.40
2013	17.01	15.98	16.74	16.00
2014	16.43	15.64	15.89	15.10
2015	15.99	14.81	15.61	14.94

Shawnigan Lake and Wind River fertilization trials (McWilliams and Thérien, 1997; Brooks and Coulombe, 2009; Brooks and Mitchell, 2011). It may be significant to note that growth responses also demonstrated a lag in regional networks of fertilization trials established across British Columbia (Omule, 1990) and western Washington and Oregon (Stegemoeller and Chappell, 1991). However, the average lag observed across these latter networks containing a wide range of sites may mask more site-specific patterns. In addition, the apparent lack of detected growth lags may be an artifact of remeasurement intervals on permanent plots. In regard to the former, site index at Shawnigan Lake and Wind River was quite low (21 m and 18 m at 50 years, respectively) and analyses of regional networks in British Columbia, Oregon, and Washington focused on only the average timing of growth response across a wide range in site indices (Omule, 1990; Hann et al., 2003). For example, site index ranged from 16 to 46 m at 50 years for individual installations in the Regional Forest Nutrition Research Project (RFNRP) (Peterson and Hazard, 1990), from 17 to 37 m at 50 years in the British Columbia fertilization trials (Omule, 1990), and from 17 to 48 m at 50 years in an analysis of a comprehensive dataset from British Columbia, Washington, and Oregon (Hann et al., 2003).

As alluded to above, conclusions about any lag in growth responses are complicated by variation in the length of growth periods among different studies. A comprehensive statistical analysis of direct fertilization responses in Douglas-fir indicated an immediate response followed by a gradual decline lasting up to 15 years (Hann et al., 2003). However, this analysis was based on growth periods that ranged in length from two to five years, limiting the opportunity to detect lags of one year, and probably even lags of two or three years given the potential influence of 4- or 5-yr growth periods in the analysis (Hann et al., 2003). In one analysis involving only 5-yr growth periods, the maximum direct response of Douglas-fir to fertilization was concluded to occur in the first 5-yr period after fertilization (Wang, 1990). In contrast, 2-yr remeasurements led to the conclusion that growth response peaked between the 2nd and 4th growing season after fertilization (Miller et al., 1989). Retrospective analyses based on annual growth rings offer the advantage of being able to identify the specific year in which the direct basal area growth response peaks. For example, Brix and Mitchell (1980) found a gradual increase in radial increment relative to unfertilized trees through at least the first four years after fertilization (69%, 113%, 102%, and 131% increase, respectively). Statistical modeling of direct growth responses to fertilization, however, generally has assumed no interaction with annual climatic fluctuations. For large datasets covering many sites that are fertilized in many different years, this assumption is probably realistic for describing past trends, but climatic fluctuations almost certainly have increased the variability in these trends over time since fertilization. On individual field trials interactions with climate have been shown to strongly influence annual growth responses (e.g., Lim et al., 2015).

Isotopic composition of growth rings provides a unique tool for concurrent assessment of annual trends in direct basal area growth response, the decline in $\Delta^{13}C$, and the increase in *i*WUE. Systematic changes in the timing of growth responses and the relative importance of *i*WUE along a gradient in site quality may lead to insights into the mechanisms by which growth responds to N fertilization. Brix (1983) assessed the relative contribution of growth efficiency and total leaf area with increasing time since fertilization, with the former peaking several years after fertilization and then diminishing to near zero by year five. Foliage mass increased steadily to a plateau approximately six years after fertilization, although the implied increase in intercepted PAR was not addressed. Other

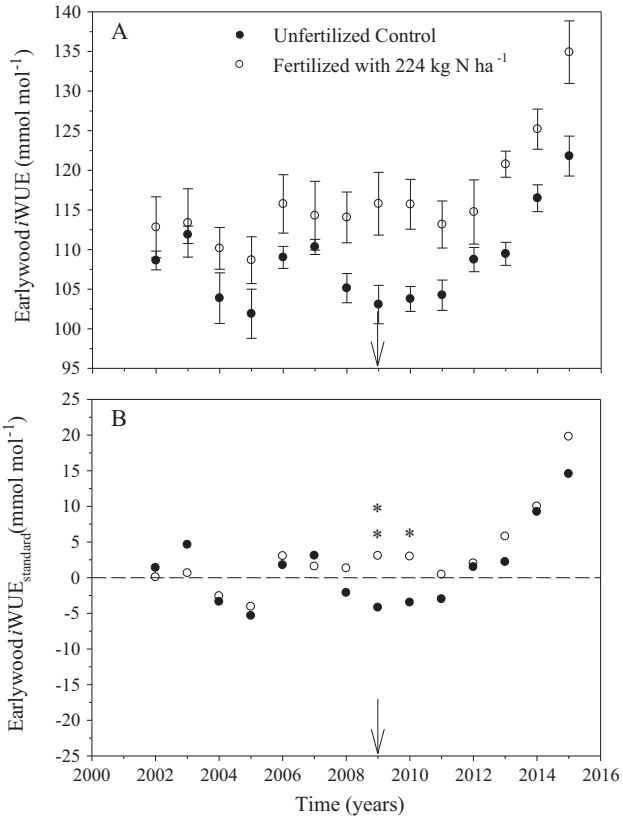

Fig. 4. (A) Mean intrinsic water use efficiency (iWUE) with standard errors, and (B) least square means of the standardized intrinsic water use efficiency (iWUEstandard) of earlywood in unfertilized control trees and trees fertilized with 224 kg N ha^{-1} in the STT replication of the Giustina Fertilization Trial (n = 6 trees per treatment). The arrow indicates when the fertilizer was applied. * = significant effect $\alpha = 0.05$; ** = highly significant effect $\alpha = 0.01$.

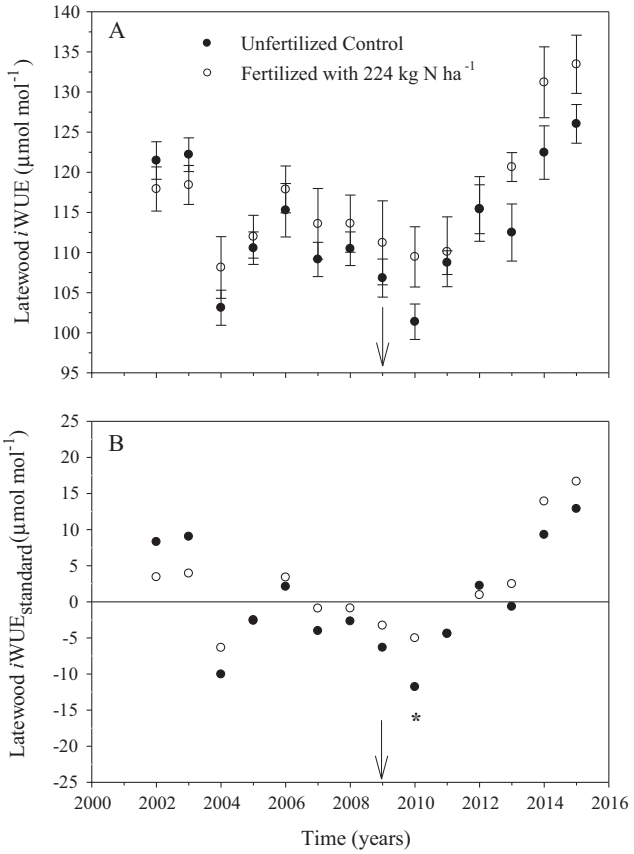

Fig. 5. (A) Mean intrinsic water use efficiency (iWUE) with standard errors, and (B) least square means of the standardized intrinsic water use efficiency (iWUEstandard) of latewood in unfertilized control trees and trees fertilized with 224 kg N ha^{-1} in the STT replication of the Giustina Fertilization Trial (n = 6 trees per treatment). The arrow indicates when the fertilizer was applied. * = significant effect $\alpha = 0.05$.

studies have demonstrated the importance of a shift in carbon allocation from belowground to aboveground components as a third mechanisms of stem growth responses to fertilization (e.g., Axelsson and Axelsson, 1986), and results of some studies suggest this reallocation is the major mechanism driving fertilization response (Gower et al., 1994; Lim et al., 2015). By contrast, release from pathogen-induced carbon limitation has been shown to greatly stimulate stem growth of Douglas-fir in this region (Saffell et al., 2014), and a follow-up study found that this release from pathogen pressure prompted greater proportional increases in coarse root biomass increment compared to stem growth increment (Voelker et al., unpublished dataset). Therefore, assuming N-fertilization does decrease allocation to fine roots to some extent, gains in total canopy carbon gain may not always be expected to greatly change the ratios of above versus below-ground carbon allocation.

Some of the response to N applications may result from stimulation of mineralization in the typically large pool of otherwise unavailable N on many sites (Aber et al., 1993; Contosta et al., 2011), but the N from this source would likely contribute to the first two mechanisms involving growth per unit leaf area and increasing total leaf area. Douglas-fir trees have been shown to continue responding to long-term net mineralization of organic matter stimulated by N fertilization, and that even the net amount represents a small portion of gross mineralization due to concurrent immobilization by decomposers (Strader and Binkley, 1989).

The response of $\Delta^{13}C$ to N fertilization starts to provide some insight into the ecophysiological mechanisms inducing the immediate growth responses described above. Nitrogen fertilization at the conventional rate for Douglas-fir management (224 kg N ha^{-1}) was shown to significantly reduce earlywood and latewood $\Delta^{13}C$ at STT. At Wind River, a site of much lower site quality, Brooks and Coulombe (2009) were able to evaluate fertilization effects on $\Delta^{13}C$ at three different rates of N application (157, 314, 417 kg ha^{-1}), the lowest being 30% lower than the rate applied at STT and the two highest being 40% and 86% more than applied at STT. Despite the differences in N application rates and site productivity, the reductions in earlywood $\Delta^{13}C$ of 0.7 and 0.6‰ at STT in the first (2009) and second (2010) growing seasons after the fertilization, respectively, were similar but slightly less than the reduction in earlywood $\Delta^{13}C$ found by Brooks and Coulombe (2009) in the first two growing seasons after fertilization (1964 and 1965). Variation in N application rates, site productivity, and/or annual climatic conditions, however, probably explain why the reduction in latewood $\Delta^{13}C$ of 0.6‰ in the second growing season at STT was about half of the 1.4‰ reduction at Wind River. The difference in *i*WUE response to fertilization between Wind River and the Giustina Trial at STT may indicate the more productive conditions at STT, where leaf nitrogen was likely higher prior to fertilization than at Wind River. If pre-treatment leaf nitrogen concentration was in fact higher at STT, a smaller increase in photosynthesis and *i*WUE would be expected, assuming stomatal conductance remained somewhat similar before and after fertilization.

The response of $\Delta^{13}C$ and *i*WUE at STT also differed in both magnitude and timing from those reported for Shawnigan Lake (Brooks and Mitchell, 2011). Fertilization with 448 kg N ha^{-1} at

Shawnigan reduced $\Delta^{13}C$ and increased *i*WUE for a period that was twice as long (four years) as at STT. Compared to Shawnigan Lake, the STT site had about half the N-fertilization rate and twice the inherent productivity. These differences probably explain the limitation of a fertilization effect to only two years in the earlywood and one year in the latewood at STT, compared to four years in both at Shawnigan Lake. Differences in climate conditions during the treatment period could also contribute to differences in fertilization responses found in these different studies.

Stomatal conductance and water-use efficiency can be expected to be affected by soil water availability and climatic influences such as vapor pressure deficit (Leverenz, 1981a, 1981b; Meinzer, 1982; Voelker et al., 2014). Fluctuations in temperature and in precipitation have been shown to significantly influence Scots pine NPP and responses to fertilization, respectively (Lim et al., 2015). The climate responsiveness of this one-time fertilization trial cannot be compared directly to Lim et al. (2015) because their fertilization occurred annually over an eight-year period. Nonetheless, comparisons of inter-annual variation in $\Delta^{13}C$ to monthly PRISM meteorological data (http://www.prism.oregonstate.edu) indicated that compared to the control stands the N-fertilized stands tended to have enhanced sensitivity of latewood $\Delta^{13}C$ to early growing season (i.e. May, June and July mean) maximum temperatures ($r = -0.43$ and -0.70, respectively) and vapor pressure deficit ($r = -0.47$ and -0.75, respectively). Similar to the patterns noted in an earlier study of fertilization effects on Douglas-fir (Brooks and Coulombe, 2009), these large differences in correlation coefficients, and perhaps the greater ratio of earlywood to latewood widths in fertilized trees, suggest that fertilization-induced increases in leaf area and associated increases in maximum transpiration rates may deplete soil water earlier in this summer-dry environment. This effect would be expected to constrain the total potential carbon gain from changes in *i*WUE during the late growing season when LW cells form. However, as implied by growth patterns at STT and at Shawnigan Lake (Brix and Mitchell, 1980), increased earlywood growth in response to fertilization can increase the earlywood:latewood ratio even in the same years that latewood growth responds significantly. A formal set of statistical analyses designed to discern how climate variability interacts with silvicultural treatments like fertilization to affect leaf gas exchange and allocation will require longer time-series of concurrent tree-ring growth, $\Delta^{13}C$, and ^{18}O (Scheidegeer et al., 2000; Saurer and Siegwolf, 2007; Brooks and Coulombe, 2009; Barnard et al., 2012; Roden and Farquhar, 2012), as well as repeated above and belowground biomass estimates.

5. Conclusions

The effect of N fertilization on seasonal patterns of BAI and *i*WUE were reconstructed by measuring growth and canopy-integrated carbon isotope discrimination ($\Delta^{13}C$) recorded in earlywood and latewood at a replicate of the Giustina Fertilization Trials on a high quality site. The fertilizer-induced growth response appears proportional to the reduction in $\Delta^{13}C$ and therefore largely attributable to the implied increase in *i*WUE. Although knowledge of potential shifts from belowground to aboveground biomass allocation would confirm the relative contributions of alternative or concurrent response mechanisms, the greater carbon fixation due to increased *i*WUE and leaf area in this study alleviate the need to invoke changes in allocation to account for stem growth responses. Previous studies of N fertilization of Douglas-fir forests have reported increased growth and *i*WUE but were limited to very low productivity sites and relatively high fertilization rates. This detailed study of the STT installation of the Giustina Fertilization trials has shown that Douglas fir plantations on highly productive sites can also respond positively to more moderate (operational) levels of N fertilization. Although the current operational strategy is to fertilize medium to low quality sites to achieve a higher frequency of response, the challenge ahead is to develop diagnostic criteria for *a priori* detection of response potential on relatively high quality sites that have historically demonstrated high response variability in regional fertilization trials (Peterson and Hazard, 1990).

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