

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 to increase growth rates. Understanding the mechanisms of response facilitates prioritization of stands for treatment. The 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 ($\Delta^{13}\text{C}$) 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 interaction effect between fertilization treatment and year was found for total basal area growth and earlywood basal area increment. Fertilized trees showed significant 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 fertilization effect was found for latewood basal area increment only in the first growing season after treatment. A significant interaction effect was also found for $\Delta^{13}\text{C}$ and *i*WUE in earlywood and latewood. Fertilization significantly reduced earlywood $\Delta^{13}\text{C}$ and increased earlywood *i*WUE in the first and second growing seasons after fertilization. Only a marginally significant fertilization effect was detected for latewood $\Delta^{13}\text{C}$ and *i*WUE in the second growing season after treatment. Previous studies of nitrogen fertilization of Douglas-fir forests have reported increased growth and *i*WUE at very low productivity sites and at relatively high fertilization rates. This study has shown that Douglas fir

plantations on highly productive sites can also respond positively to more moderate (operational) levels of nitrogen fertilization, but the duration of growth response appears shorter.

Keywords

Fertilization, *Pseudotsuga menziesii*, basal area, carbon discrimination, water use efficiency

Introduction

Nitrogen is the most important limiting nutrient in terrestrial ecosystems in most of the world (Field and Mooney 1986, LeBauer and Treseder 2008), including the Pacific Northwest region (PNW) of the U.S. 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 (N) can limit plant growth on a given site for several reasons: 1) small nitrogen pools; 2) large nitrogen pools but low availability; 3) rapid leaching of available nitrogen 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 nitrogen to optimal physiological functioning of plants is directly related to its role as a fundamental component of amino acids, proteins, nucleic acids, and enzymes (including Rubisco) in a plant, 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 nitrogen, a positive relationship between photosynthetic rate and leaf nitrogen content has been reported for a wide variety of plant species including Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) (Brix 1981a, Field and Mooney 1986, Mitchell and Hinckley 1993, Ripullone et al. 2003, Manter et al. 2005). The limitation that nitrogen

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 nitrogen concentrations of 1.74 % shortly after fertilization enhanced the photosynthetic rate to approximately 30% greater than that measured at a leaf nitrogen concentration of 1.0 % in unfertilized plots (Brix 1981b). 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 1981a). 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 a longer term phase of elevated leaf area index (Brix 1983).

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 nitrogen fertilization at Shawnigan Lake and on larger plot networks in the Douglas-fir region have led to widespread operational nitrogen applications in Douglas-fir (Peterson and Hazard 1990), amounting to 50,000 and 55,000 ha treated annually in the late 1980s through the 1990's (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 fertilization by applying N to only

those stands or sites that can both 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. More 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 fertilization at a scale directly transferrable to operational fertilization prescriptions (Brooks and Coulombe 2009, Brooks and Mitchell 2011).

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 well as physiological responses such as stomatal conductance. Therefore, the carbon stable isotopic composition of C_3 plant tissues is often expressed as carbon isotope discrimination ($\Delta^{13}\text{C}$), 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 ($i\text{WUE}$), 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. 2016). 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). 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 nitrogen fertilization (157, 314, 417 kg ha⁻¹) in an 85 year-old Douglas-fir plantation located at the Wind River Experimental Forest, 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 nitrogen fertilization, $\Delta^{13}C$ was reduced and $iWUE$ was increased in both earlywood and latewood components, but only for the first three years before returning to pretreatment levels. They interpreted this short-term $\Delta^{13}C$ response to an increase in leaf nitrogen and photosynthesis, while the longer-term growth response was attributed to an increase in leaf area. Brooks and Mitchell (2011) found similar responses to nitrogen 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 nitrogen 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 nitrogen and photosynthesis, 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 a decrease in $\Delta^{13}C$ in fertilized Douglas-fir plantations in the interior northwestern USA, despite a significant

growth response to treatment. They speculated that their relatively coarse 3-yr growth period may prevent detection of shorter-term response of $\Delta^{13}\text{C}$, and that increases in leaf area were largely responsible for the increases in growth.

Earlier work studying growth, $\Delta^{13}\text{C}$, and $i\text{WUE}$ responses to nitrogen fertilization in Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) forests was generally conducted at 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 nitrogen fertilization. Of particular interest was the degree of consistency in mechanisms at a site 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 are providing detailed description of crown structural responses to fertilization, including estimates of total leaf area, and is currently ongoing. The objective of the analysis reported here was to test the hypothesis that fertilization reduced $\Delta^{13}\text{C}$ and increased $i\text{WUE}$, implying that an increase in foliar nitrogen concentration had greater relative impact on A than g_s . The mechanisms identified in the Giustina Trials will be incorporated into an individual-tree growth model expanded from an ORGANON variant developed for intensively managed Douglas-fir plantations (Hann et al. 2003).

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 last 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 nitrogen 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 Starker Forests installation (STT) of the Giustina Fertilization Trials was selected as an intensive study site to test the effect of fertilization on $\Delta^{13}\text{C}$ and $i\text{WUE}$ in a relatively high productivity stand on a relatively moist site. This replicate is located at 44° 34' 39" North latitude and -123° 29' 34.7994" West longitude at an elevation of 283 m. Site index was 46 m at 50 years (King 1966), and 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>).

Tree measurements and wood sample collection

In November 2015, three trees were randomly selected from each of the four measurement plots, including two control plots and two fertilized plots, yielding a total sample of 12 trees at the STT site. 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 a 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.

Tree-ring measurements and basal area increment estimation

Tree-ring widths (earlywood, latewood and whole ring measurements) 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 entire 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) ($\text{mm}^2 \text{ year}^{-1}$) 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):

$$[1] \quad DIB_{15} = 25.4 \cdot \left[0.924443655 \cdot \left(\frac{DOB_{15}}{2.54} \right)^{0.988866545} \cdot e^{[-0.034145503 \cdot (1-CR)^{0.5}]} \right]$$

where DIB_{15} was estimated diameter inside bark at breast height (cm) and DOB_{15} was DBH in 2015 (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 earlywood and latewood conformed to a circle each year.

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

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

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

$$[3] \quad \Delta^{13}\text{C} = \frac{\delta^{13}\text{C}_{\text{air}} - \delta^{13}\text{C}_{\text{cell}}}{1 + \delta^{13}\text{C}_{\text{cell}}/1000}$$

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

Following Farquhar et al. (1982, 1989) $\Delta^{13}\text{C}$ 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:

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

where a is the fractionation of 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 to estimate intrinsic water use efficiency ($iWUE$) as:

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

where A is assimilation, g_s is stomatal conductance and the constant 1.6 represents the ratio of stomatal conductance for CO₂ to water vapor (Osmond et al. 1980, Farquhar et al. 1989, p. 3-8 in Ehleringer et al. 1993; Seibt et al. 2008).

Statistical analysis

The responses $\Delta^{13}\text{C}$ and $iWUE$ were standardized to account for pre-treatment differences (Brooks and Mitchell 2011). For both $\Delta^{13}\text{C}$ and $iWUE$, 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 nitrogen fertilization effects was as follows:

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

where: y_{ijk} = total ring basal area increment (TOTBAI), earlywood basal area increment (EWBAI), latewood basal area increment (LWBAI), standardized $\Delta^{13}\text{C}$, or standardized $iWUE$ 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 ($\text{m}^2 \text{ha}^{-1}$) 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.

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, Littell et al. 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, $\Delta^{13}\text{C}$, 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).

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 \times year interactions were detected for TOTBAI ($p = 0.0005$), EWBAI ($p = 0.0003$), and LWBAI ($p = 0.0255$). Initial basal area as a covariate was not significant ($p = 0.651$, 0.454 , and 0.175 , respectively) so was eliminated from the model. Likewise, 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) (Figure 1). Nitrogen fertilization also had a significant effect on EWBAI during the first and second growing seasons after application ($p = 0.0150$ and $p = 0.0109$, respectively) (Figure 2). In contrast, nitrogen fertilization had a significant effect on LWBAI in only the first growing season after application ($p=0.0466$; Figure 3). Compared to TOTBAI and EWBAI, fertilization effects on LWBAI were also not as robust (lower p-values).

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, nitrogen-fertilized trees had significantly lower $\Delta^{13}\text{C}$ 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, nitrogen-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) (Figure 4). In the latewood, nitrogen-fertilized trees had significantly lower $\Delta^{13}\text{C}$ ($p = 0.0159$) (Table 4) and *i*WUE ($p=0.0154$) than the control trees only in the second growing season after treatment application (Figure 5).

Fertilization reduced earlywood $\Delta^{13}\text{C}$ by 1.2 and 1.1 ‰ compared to the control trees in 2009 and 2010, respectively; corresponding to an increase of 12.3 and 11.5 % in *i*WUE compared to the control trees. Fertilization reduced latewood $\Delta^{13}\text{C}$ by 0.75 ‰ compared to the control trees, representing an 8% increase in *i*WUE during the 2nd growing season.

Discussion

Nitrogen fertilization applied at operational levels for managed forests of western Oregon and Washington (224 kg N ha^{-1}), increased both growth and *i*WUE of a young Douglas-fir plantation that was part of a regional study to elucidate mechanisms of growth response to fertilization. Growth responses were significant in both the earlywood and latewood, 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, which were strongest in the first and second year after the fertilizer was applied. The timing of fertilization

effects on *i*WUE was broadly consistent with previous research on fertilization of Douglas-fir forests (e.g., Brooks and Coulombe 2009, Brooks and Mitchell 2011). The direct effects of fertilization on growth were a year earlier relative to the one-year lag in low productivity stands at the Shawnigan Lake and Wind River field trials (McWilliams and Thérien 1997, Brooks and Coulombe 2009, Brooks and Mitchell 2011), in extensive networks of regional fertilization trials in British Columbia (Omule 1990), and in the RFNRP (Regional Forest Nutrition Research Project) network in Washington and Oregon (Stegemoeller and Chappell 1991).

The influence of site quality on the temporal pattern of growth responses is unclear, but one possibility is that the response is more immediate and ends earlier where site index is greater. Site index at Shawnigan Lake and Wind River was quite low (21 m and 18 m at 50 years, respectively) and analyses of the regional networks focused on only the average responses for a range in site indices (e.g., range of 16 to 46 m at 50 years for individual installations in the RFNRP network as described by Peterson and Hazard (1990)). A comprehensive analysis of direct fertilization responses in Douglas-fir indicated an immediate response followed by a gradual decline lasting up to 15 years, with relative magnitude increasing with decreasing site index (Hann et al. 2003). However, the resolution of the growth periods for repeated measurements of permanent plots (2-5 years) limits the opportunity to test for temporal patterns on an annual scale (Hann et al. 2003), in contrast to the annual resolution of increment core measurements. The difference in apparent temporal trends in growth response appear strongly dependent on growth period lengths, with 2-yr growth periods suggesting a gradual increase to a peak between the 2nd and 4th growing season after fertilization (Miller et al. 1988), and 5-yr growth periods suggesting a maximum in the first 5-year growth period after fertilization followed by a gradual decline (Wang 1990). Regardless, annual growth rings at Shawnigan Lake

and Wind River suggested a 2-3-yr rise to a peak, in contrast to an immediate peak during the growing season after fertilization at the Starker installation of the Giustina Fertilization Trials. Future work will be needed to test the possibility that the temporal trend in growth response to fertilization differs among sites of differing types or quality. The mechanistic reason for such a difference may be important for discriminating between responding and non-responding sites. For example, some of the response to nitrogen applications may result from stimulation of mineralization in the typically large pool of otherwise unavailable nitrogen on many sites (Aber et al. 1993, Contosta et al. 2011). Douglas-fir trees have been shown to continue responding to long-term net mineralization of organic matter stimulated by nitrogen fertilization, but even the net amount has been demonstrated to represent a small portion of gross mineralization due to concurrent immobilization by decomposers (Strader and Binkley 1989). The rapid pre-fertilization tree growth on higher quality sites may facilitate a more rapid draw on the nitrogen from the fertilizer due to its readily available form, stimulating a more immediate but brief direct effect on growth.

Nitrogen fertilization has been widely reported to increase growth in Douglas-fir stands and plantations (Gessel and Walker 1956, Gessel and Shareeff 1957, Chappell et al. 1991, Mainwaring et al. 2014). Brix and Mitchell (1980) reported that nitrogen fertilization at a rate of 448 kg ha⁻¹ in a 24 year-old stand of Douglas-fir located at Shawnigan Lake, Vancouver Island, BC, induced a 201 % increase in the ring width relative to unfertilized controls for the first four years after application. In the STT installation of the Giustina Fertilization Trial, 224 kg N ha⁻¹ increased TOTBAI in only the first and second growing seasons after application. A general negative relationship between site index and relative basal area growth response to nitrogen fertilization has previously been reported (Webster and Farnum, 1980, Miller et al.

1986, Miller et al. 1989, Carter and Klinka 1992, Carter et al. 1998). However, the relative basal area growth response reported at the STT installation of the Giustina Trials underscores the variability around this general trend and was more similar to that reported by Brix and Mitchell (1980), despite the fact that site quality was dramatically higher at STT (site index 46 m at 50 years in contrast to 21 m at 50 years at Shawnigan) (King 1966). Furthermore, application of nitrogen fertilizer to medium and high productivity sites like STT may give higher financial returns if the absolute volume increase is greater (Carter et al. 1998), underscoring the importance of predicting response to nitrogen application using criteria that complement site index.

Concerns have often been expressed about fertilization effects on wood quality, particularly with respect to potential impact of accelerated growth rates on wood density. Because wood density is controlled largely by the relative proportions of latewood to earlywood, it is important to understand fertilization effects on EWBAI and LWBAI. EWBAI and LWBAI increased significantly for twenty years at the two highest fertilization rates of 314 and 417 kg ha⁻¹ at Wind River (Brooks and Coulombe 2009). In the STT replicate of the Giustina Trials, TOTBAI and EWBAI of fertilized trees were significantly greater for only the first two growing seasons, as was LWBAI in only the first growing season. However, most of the increase in growth was in the earlywood, thus increasing the early to latewood ratio. Given that the Giustina trials have accrued seven growing seasons since treatment, the direct effects on TOTBAI and EWBAI growth responses appear to have persisted only about two years under the more common operational rate of 224 kg N ha⁻¹ and under conditions of higher site quality (site index of 46 m at 50 years).

In a study expanded to include responses to both thinning and fertilization provided by the long-term study at Shawnigan Lake, Brooks and Mitchell (2011) analyzed tree-rings in increment cores to test not only fertilization effects but also thinning and interaction effects over an 18-year period following treatment of the 41-year-old plantation in 1971. Fertilization with 448 kg N ha⁻¹ had a significant effect ($\alpha = 0.01$) on EWBAI for six years after application (1971 to 1976). Similar results were observed for TOTBAI, except for the lack of a significant response in 1971. In contrast, the fertilization effect on LWBAI was significant only in 1974 and 1975. The fact that the high quality STT site was treated at half the fertilization rate (224 kg N ha⁻¹) may explain some of the differences in its response to the fertilization treatment.

The response of $\Delta^{13}\text{C}$ to nitrogen 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 $\Delta^{13}\text{C}$ at STT. At Wind River, a site of much lower site quality, Brooks and Coulombe (2009) were able to evaluate fertilization effects on $\Delta^{13}\text{C}$ at three different rates of nitrogen 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 nitrogen application rates and site productivity, the reductions in earlywood $\Delta^{13}\text{C}$ of 1.2 and 1.1 ‰ at STT in the first (2009) and second (2010) growing seasons after the fertilization, respectively, were similar to the reduction in earlywood $\Delta^{13}\text{C}$ found by Brooks and Coulombe (2009) in the first two growing seasons after fertilization (1964 and 1965). Differences in nitrogen application rates and/or site productivity, however, probably explain why the reduction in latewood $\Delta^{13}\text{C}$ of 0.75 ‰ in the second growing season at

STT was about half of the 1.4 ‰ reduction at Wind River, and lasted for a shorter time than at Wind River.

Nitrogen fertilization induced corresponding increases in *i*WUE during formation of both earlywood and latewood at STT, similar to those reported by Brooks and Coulombe (2009). Specifically, nitrogen fertilization increased *i*WUE during earlywood formation in fertilized trees by 12.3 and 11.5 ‰ relative to control trees in the first and second growing season after application, respectively. Brooks and Coulombe (2009) reported that *i*WUEs inferred from wood formed in fertilized trees were 20 ‰ greater than in control trees during the first and second growing seasons after the fertilization. The difference in *i*WUE response to fertilization between Wind River and the Giustina Trial at STT may indicate that the moister conditions in coastal Douglas-fir forests allowed stomatal conductance to increase concurrently but to a lesser degree with photosynthesis, causing a smaller response in *i*WUE.

In contrast to Wind River (Brooks and Coulombe (2009)), the response of $\Delta^{13}\text{C}$ and *i*WUE at STT differed in both magnitude and timing from those reported for Shawnigan Lake (Brooks and Mitchell 2011). Despite the similarly low productivity (site index 21 m at 50 years) but possibly wetter conditions at Shawnigan relative to Wind River, Brooks and Mitchell (2011) reported that fertilization with 448 kg N ha⁻¹ at the latter site reduced $\Delta^{13}\text{C}$ and increased *i*WUE for a period that was twice as long (four years). In STT earlywood, maximum $\Delta^{13}\text{C}$ reductions of 1.2 and 1.1 ‰ during the first and second growing seasons, respectively, were similar to the reduction of 1.5 ‰ reported for Shawnigan Lake. However, in STT latewood, the maximum $\Delta^{13}\text{C}$ reduction of 0.75 ‰ in fertilized trees amounted to only half the reduction of 1.5 ‰ observed at Shawnigan Lake. Corresponding increases in earlywood *i*WUE of 12.3 and 11.5 ‰ associated with nitrogen fertilization at STT in the first and second growing seasons, were lower

than the earlywood range (15-20 %) reported by Brooks and Mitchell (2011) for four years following fertilization at Shawnigan. Furthermore, the 8% increase in *i*WUE implied by latewood at STT was detected only in the second growing season at STT. Compared to Shawnigan Lake, the STT site had about half the nitrogen-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.

The effect of N fertilization on tree growth has been broadly documented for Scandinavian tree species (Tam 1991, Hyvönen et al. 2008), for loblolly pine in the southeastern U.S. (Fox et al. 2007, Carlson et al. 2014), and for Douglas-fir in the PNW (Brix 1993, Brooks and Coulombe 2009, Brooks and Mitchell 2011, Mainwaring et al. 2014). In our study, we detected a significant fertilization effect on basal area growth of treated trees with 224 kg N ha⁻¹ at a highly productive site in the first two growing seasons. We speculate that N fertilization boosted leaf N concentration, enhanced photosynthetic processes (Brix 1981a), and reduced $\Delta^{13}\text{C}$ and increased *i*WUE during earlywood formation. Assuming the higher foliar N reflected a greater concentration of photosynthetic enzymes and an enhanced carboxylation process, more rapid utilization of C would be expected to reduce $\Delta^{13}\text{C}$ and increase *i*WUE, assuming stomatal conductance did not also increase. Although this process appears to be a relatively short-term response to nitrogen fertilization, the longer-term response of increasing leaf area index has been documented (Brix 1981b, Brix 1983). In fact, at STT we detected a significant N fertilization effect on several branch attributes for at least three growing seasons after the fertilizer was applied in 2009 (Mainwaring et al. 2013). Trees treated with 224 kg N ha⁻¹ exhibited significantly greater three-year-old foliage mass in the second growing season (2010) after

fertilization, greater terminal shoot growth in the first, second, and third growing seasons after fertilization and greater one-year-old lateral shoot growth in the second and third growing seasons. Furthermore, bud count was significantly greater in treated trees compared to the control trees in the third growing season after fertilization.

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, b, Meinzer 1982). At STT over the period from 2009 to 2015, the average total rainfall was 1677 mm, although 2133 mm fell in 2012 and only 994 mm fell in 2013. The maximum monthly temperature (T_{\max}) ranged from 15.4 in 2011 to 18.6°C in 2015, while the minimum monthly temperature (T_{\min}) ranged from 3.6 in 2009 to 4.9°C in 2010. The maximum monthly vapor pressure deficit (VPD_{\max}) averaged 1.1 kPa, ranging from 0.98 in 2011 to 1.4 kPa in 2015 (<http://www.prism.oregonstate.edu>). However, during the summer drought period (June through September), rainfall, T_{\max} , T_{\min} , and VPD_{\max} were completely different. Interestingly, the lowest annual rainfall was recorded in 2013, the year that also experienced the highest summer rainfall (262 mm). The highest daily T_{\max} (27.3 °C) and VPD_{\max} (2.6 kPa) during the post-fertilization growth period occurred in 2015, and the second highest VPD_{\max} (2.3 kPa) occurred in 2014. These years corresponded to the sixth and seventh growing seasons after fertilization, long after any significant fertilization effect was detected on TOTBAI and EWBAI, or on $\Delta^{13}\text{C}$ or $i\text{WUE}$, although it did have the highest $i\text{WUE}$ measured for the study period in both control and fertilized stands for both early and latewood. Stomatal conductance is reduced considerably when the vapor pressure deficit approaches a range of 2.0 to 3.0 kPa, and stomata are nearly closed at values above 3.5 kPa, negatively affecting transpiration and CO_2 diffusion in Douglas-fir (Leverenz 1981a, b, Meinzer 1982). The environmental conditions described indicated that

T_{\max} and VPD_{\max} had an increasing trend during the drought season in the last two years, so may have diminished the opportunity for any continued indirect growth response attributable to greater leaf area, along with the lack of any indirect response that might have been otherwise indicated by a response in $\Delta^{13}\text{C}$ and $i\text{WUE}$. This study was limited to only stable carbon isotopes, despite the recognition that a dual approach coupling stable carbon and oxygen isotopes may help to explain to the relative importance of assimilation rate and stomatal conductance (Scheidegger et al. 2000, Saurer and Siegwolf 2007, Barnard et al. 2012, Roden and Farquhar 2012). Growth responses are currently under further analysis relative to environmental conditions, and may be supplemented with analysis of $\Delta^{18}\text{O}$ in archived earlywood and latewood cellulose from the sampled growth rings.

Conclusions

The effect of nitrogen fertilization on seasonal patterns of BAI and $i\text{WUE}$ were reconstructed by measuring growth and carbon isotope discrimination ($\Delta^{13}\text{C}$) recorded in earlywood and latewood at a replicate of the Giustina Fertilization Trials on a high quality site. A significant nitrogen fertilization effect on BAI was detected in the first two growing seasons after nitrogen fertilization was implemented in early 2009. Nitrogen fertilization decreased $\Delta^{13}\text{C}$ and increased $i\text{WUE}$. Previous studies of nitrogen fertilization of Douglas-fir forests have reported increased growth and $i\text{WUE}$ but were only conducted at very low productivity sites and at relatively high fertilization rates. This detailed study of the STT Giustina Fertilization replicate has shown that Douglas fir plantations on highly productive sites can also respond positively to more moderate (operational) levels of nitrogen fertilization. Although the current 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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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.

VARIABLE	TREATMENT			
	Unfertilized Control		Fertilized with 224 kg N ha ⁻¹	
	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

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	<.0001	12.22	<.0001	5.80	<.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 ($\Delta^{13}\text{C}_{\text{standard}}$) and standardized intrinsic water use efficiency ($i\text{WUE}_{\text{standard}}$) of the earlywood and latewood in the Starker Forests replication (STT) of the Giustina Fertilization Trial.

	Earlywood				Latewood			
	$\Delta^{13}\text{C}_{\text{standard}}$ (‰)		$i\text{WUE}_{\text{standard}}$ ($\mu\text{mol mol}^{-1}$)		$\Delta^{13}\text{C}_{\text{standard}}$ (‰)		$i\text{WUE}_{\text{standard}}$ ($\mu\text{mol mol}^{-1}$)	
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	<.0001	8.91	<.0001	21.63	<.0001	19.59	<.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 ($\Delta^{13}\text{C}$) 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.

	$\Delta^{13}\text{C}$ (‰)			
	Earlywood		Latewood	
	Treatment		Treatment	
	Unfertilized Control	Fertilized with 224 kg N ha ⁻¹	Unfertilized Control	Fertilized with 224 kg N ha ⁻¹
Year				
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

Figure captions

Figure 1. (A) Mean total basal area increment (TOTBAI) with standard errors, and (B) least square means of TOTBAI in the unfertilized control and fertilized with 224 kg N ha⁻¹ treatments of the STT replication of the Giustina Fertilization Trial (n=6). The arrow indicates when the fertilizer was applied. * = significant $\alpha = 0.05$; ** = highly significant effect $\alpha = 0.01$.

Figure 2. (A) Mean earlywood area increment (EWBAI) with standard errors, and (B) least square means EWBAI in the unfertilized control and fertilized with 224 kg N ha⁻¹ treatments of the STT replication of the Giustina Fertilization Trial (n=6). The arrow indicates when the fertilizer was applied. * = significant effect $\alpha = 0.05$; ** = highly significant effect $\alpha = 0.01$.

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

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

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

Figure 1

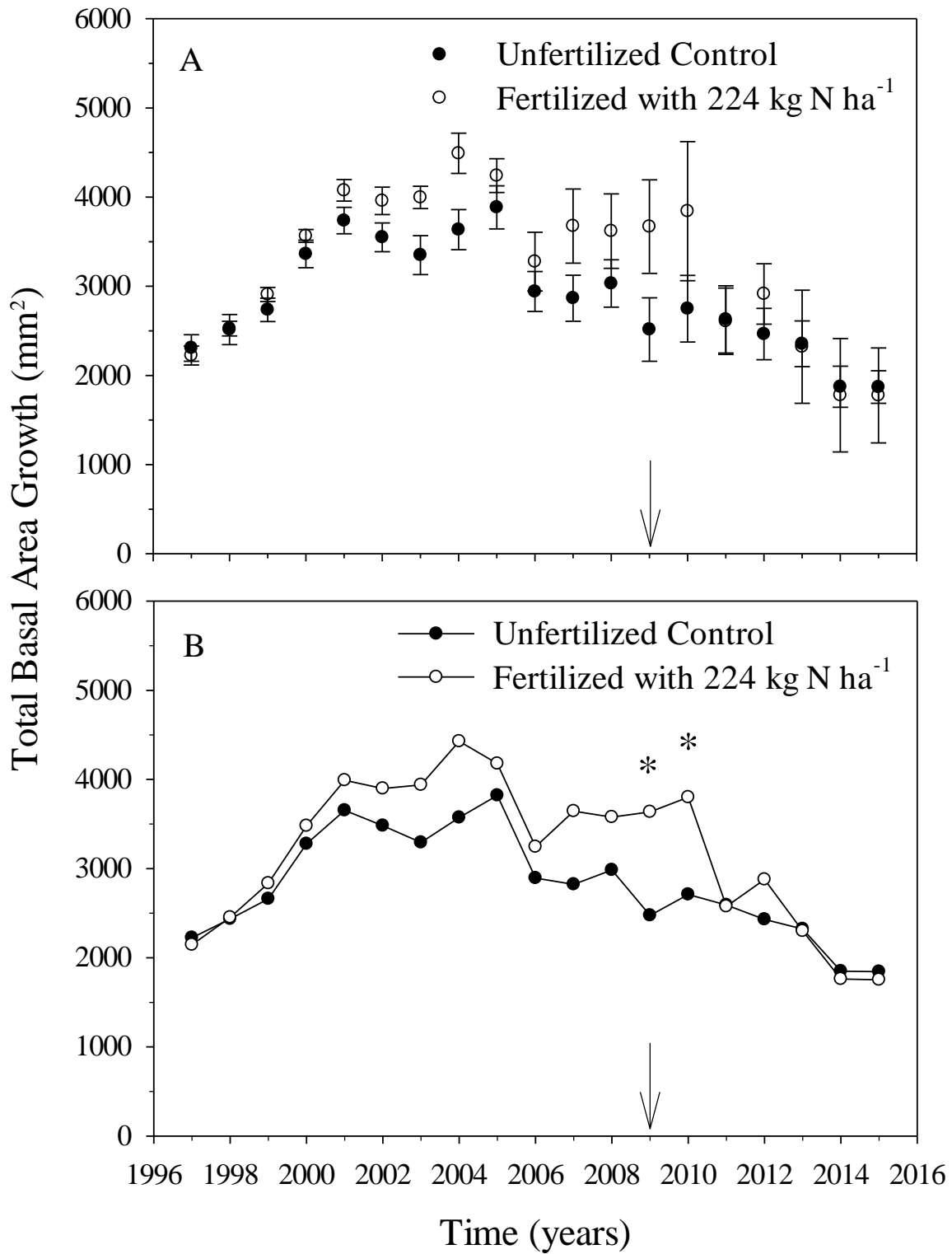


Figure 2

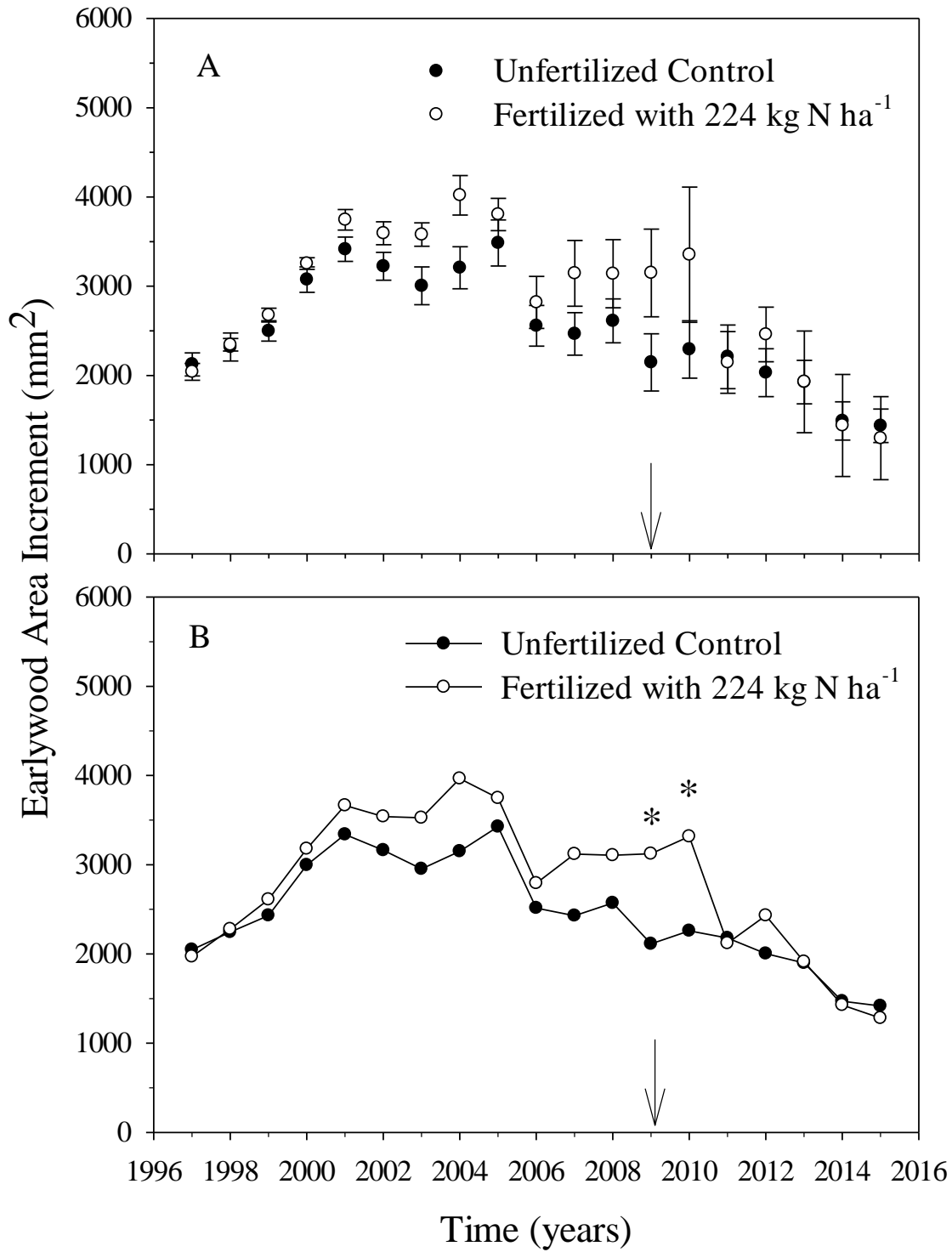


Figure 3

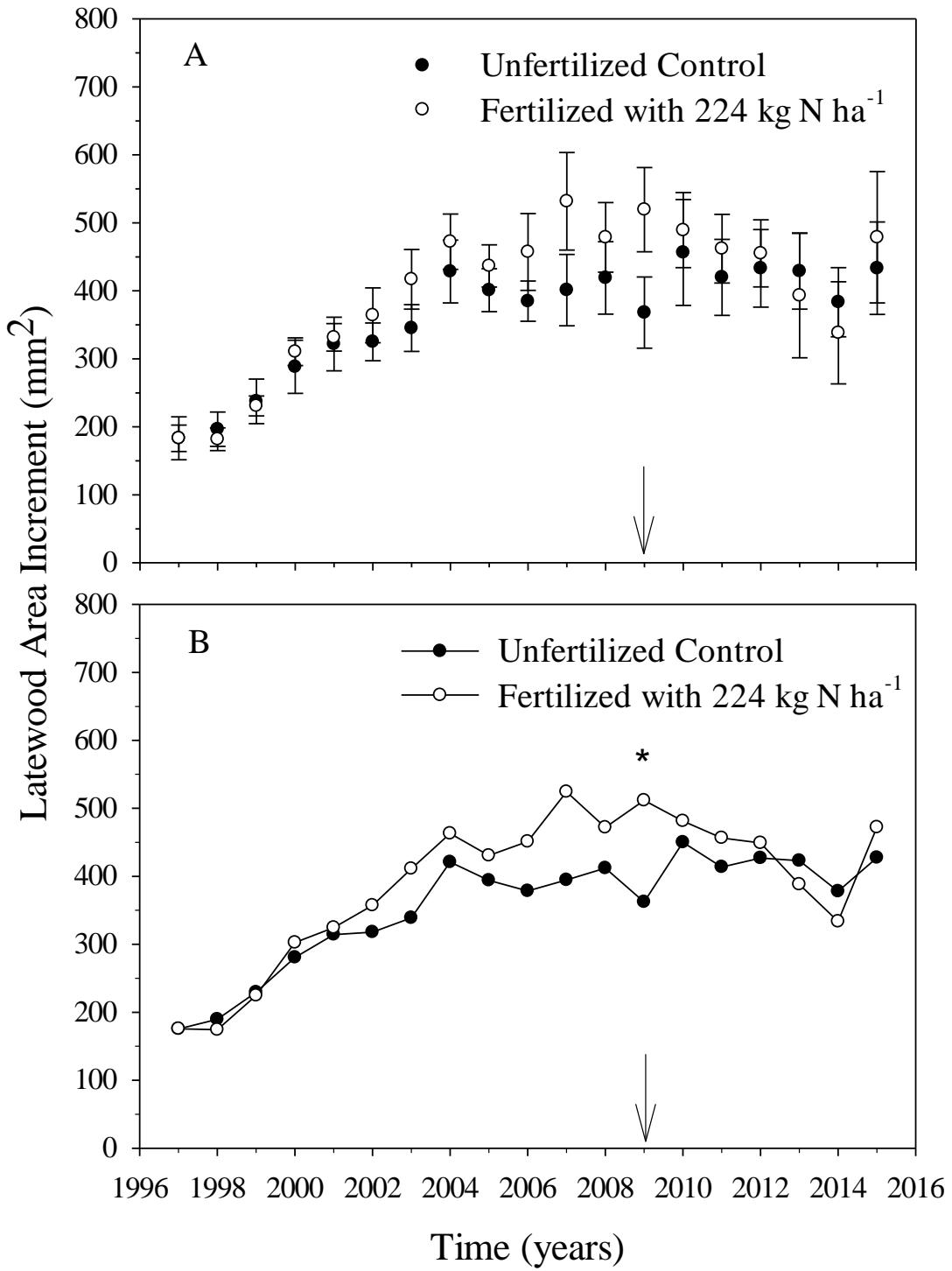


Figure 4

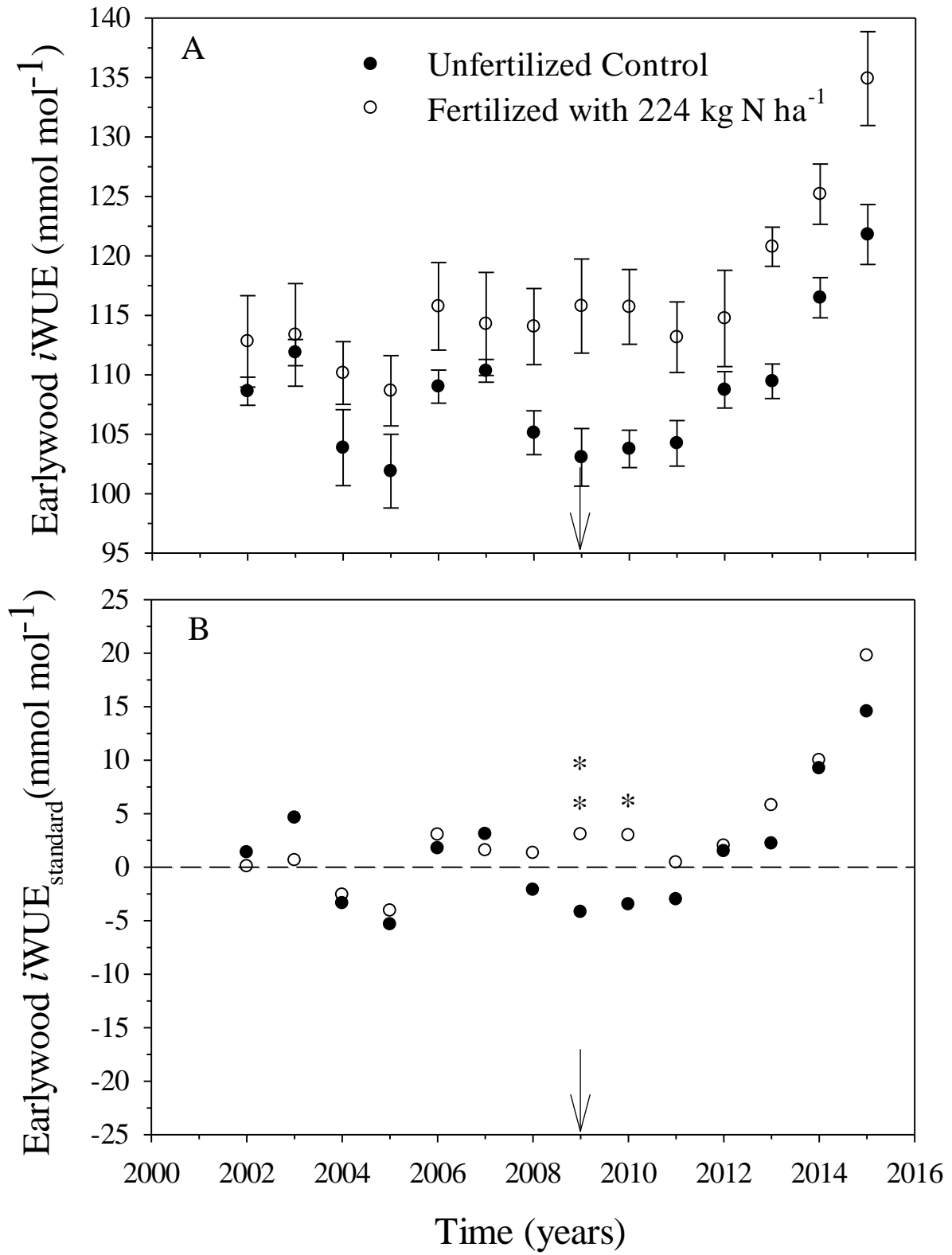


Figure 5

