

## Vegetation control effects on untreated wood, crude cellulose and holocellulose $\delta^{13}\text{C}$ of early and latewood in 3- to 5-year-old rings of Douglas-fir

Adrian Ares · Constance A. Harrington ·  
Thomas A. Terry · Joseph M. Kraft

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**Abstract** The stable carbon (C) composition of tree rings expressed as  $\delta^{13}\text{C}$ , is a measure of intrinsic water-use efficiency and can indicate the occurrence of past water shortages for tree growth. We examined  $\delta^{13}\text{C}$  in 3- to 5-year-old rings of Douglas-fir (*Pseudotsuga menziesii* (Mirb) Franco) trees to elucidate if decreased water supply or uptake was a critical factor in the observed growth reduction of trees competing with understory herb and shrub vegetation compared to those growing without competition. We hypothesized that there would be no differences in  $\delta^{13}\text{C}$  of earlywood in trees growing in plots with competing vegetation and those in plots receiving complete vegetation control during 5 years because earlywood formed early in the growing season when soil water was ample. We also hypothesized that  $\delta^{13}\text{C}$  in latewood which was formed during the later half of the growing season when precipitation was low, would be greater (less negative) in trees in plots without vegetation control. We then separated early and latewood from rings for three consecutive years and analyzed their  $\delta^{13}\text{C}$  composition. No significant differences in earlywood  $\delta^{13}\text{C}$  in years 3–5 were observed for trees in the two vegetation control treatments.

$\delta^{13}\text{C}$  of untreated latewood separated from wood cores was greater in 4- and 5-year-old rings of trees growing with competing vegetation compared to trees growing without vegetation competition (i.e.,  $-25.5$  vs.  $-26.3\text{‰}$  for year 4, and  $-26.1$  vs.  $-26.8\text{‰}$  for year 5). Results suggest that water shortages occurred in Douglas-fir trees on this coastal Washington site in the latewood-forming portion of the growing season of years 4 and 5 in the no-vegetation control treatment. We also compared  $\delta^{13}\text{C}$  from untreated wood, crude cellulose extracted with the Diglyme–HCl method, and holocellulose extracted with toluene–ethanol to see if the extraction method would increase the sensitivity of the analysis.  $\delta^{13}\text{C}$  values from the two extraction methods were highly correlated with those from untreated samples ( $r^2 = 0.97, 0.98$ , respectively). Therefore, using untreated wood would be as effective as using crude cellulose or holocellulose to investigate  $\delta^{13}\text{C}$  patterns in young Douglas-fir.

**Keywords** Carbon isotopes · Plant competition · *Pseudotsuga menziesii* · Tree rings

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A. Ares (✉)  
Forest Science, Oregon State University, 321 Richardson Hall,  
Corvallis, OR 97331, USA  
e-mail: adrian@hawaii.edu

C. A. Harrington · J. M. Kraft  
USDA Forest Service, Pacific Northwest Research Station,  
36255 93rd Avenue SW, Olympia, WA 98512, USA

T. A. Terry  
Weyerhaeuser Company, 505 N. Pearl St., Centralia,  
WA 98531, USA

### Introduction

The level of water stress necessary to reduce growth varies with plant species and genotype (Steinbrenner and Rediske 1964; Glerum and Pierpoint 1968). It has been long known that physiological performance and growth of conifer seedlings in pots can be reduced at relatively high soil water contents; e.g., soil water potential of  $-30$  kPa for *Pinus sylvestris* L. (Sands and Rutter 1959), and  $-200$  kPa for *Pinus taeda* L. (Stransky and Wilson 1964), which may be also the case for field-grown Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] (Roberts et al. 2005), a major

timber tree species in the Pacific Northwest region of the United States. Previous research has shown that in 2–3-month-old Douglas-fir seedlings growing on potted sandy soil, photosynthesis rate declined sharply after soil water potentials reached  $-100$  kPa (Zavitkovski and Ferrell 1968).

In field studies on the effects of vegetation competition on tree growth, it is difficult to identify growth limiting factors for crop trees by using the common approach of adding the limiting factor (e.g., water, nutrients). This is because addition of the limiting factor can boost growth of understory vegetation, and further suppress growth of tree crops (Roth and Newton 1996). Moreover, relevant depths for soil water content measurements are often unknown and some microsites may remain unsampled. An alternative integrated approach for assessing plant water stress is to examine the  $\delta^{13}\text{C}$  composition of plant tissues to infer possible effects of soil water supply on tree growth caused by competing vegetation. This is because decreased water supply can increase the ratio between net photosynthesis and stomatal conductance (i.e., intrinsic water-use efficiency) (Ares and Fownes 1999; Ferrio et al. 2003) often indexed by the stable carbon (C) composition ( $\delta^{13}\text{C}$ ) of plant tissues (Korol et al. 1999; Ripullone et al. 2004). Therefore,  $\delta^{13}\text{C}$  may serve as an integrated indicator of reductions in water supply (Walcroft et al. 1997; Gomez et al. 2002; Choi et al. 2005). It is important to note, however, that  $\delta^{13}\text{C}$  also relates positively to other factors such as plant nutrient status (Sparks and Ehleringer 1997).

Effects of water shortages on tree growth may be evident in the  $\delta^{13}\text{C}$  composition of tree rings formed throughout the growing season (Brooks et al. 1998; Nguyen-Queyrens et al. 1998). Although some Douglas-fir trees may exhibit second flushing, most foliage elongation ceases early in the growing season. Wood production, however, continues throughout the growing season, and thus chemical composition of foliage and wood may reflect different environmental signals (Emmingham 1977). In a tree-ring series spanning from year 10–30 in Douglas-fir stands in Vancouver Island, latewood  $\delta^{13}\text{C}$  was positively correlated with cumulative summer transpiration and soil water deficit (Livingston and Spittlehouse 1996). In that study, rings close to the pith were discarded so it was not possible to know if the relation still held for rings produced during the early years of tree development.

Separation of cellulose before determinations of  $\delta^{13}\text{C}$  in tree rings of Douglas-fir has been advocated (Livingston and Spittlehouse 1996; Marshall and Monserud 1996) because cellulose has a consistent structure and is not transferred between tree rings (Park and Epstein 1961; Tans et al. 1978). Also,  $\delta^{13}\text{C}$  is about 3‰ lower in lignin than in cellulose with the lignin to cellulose ratio varying across rings and tree stems (Wilson and Grinstead 1977)

although lignin does not migrate between rings. Therefore, the use of extracted cellulose could avoid confounding interpretations of  $\delta^{13}\text{C}$  data. In cellulose extraction, however, it is difficult to determine the endpoint of the extraction process (Harlow et al. 2006). Several approaches are followed for cellulose separation such as soxhlet extractions with toluene–ethanol followed by a bleaching step (Leavitt and Danzer 1993), or a faster techniques using acidified di-glycol methyl ether (diglyme) (MacFarlane et al. 1999). Because cellulose is commonly extracted for  $\delta^{13}\text{C}$  characterization of long tree-ring time series excluding the juvenile phase, it is unknown if using cellulose extracts is a better approach than using untreated wood for C isotope research in young coniferous trees.

The first objective of this study was to determine if decreased water supply and/or uptake was a critical factor in the growth reduction of Douglas-fir trees affected by understory herb and shrub competition. Comparing Douglas-fir trees growing in plots with and without vegetation control, we hypothesized that  $\delta^{13}\text{C}$  in latewood tissue formed during the latter half of the growing season would be higher (less negative) for trees in plots without vegetation control than for those in plots with vegetation control because they would experience intensified competition for soil water than trees in plots with vegetation control. The second objective was to determine best sampling and analytical procedures to characterize the  $\delta^{13}\text{C}$  composition in rings of young Douglas-fir trees. Towards this purpose, we examined differences in  $\delta^{13}\text{C}$  between untreated samples and cellulose (either crude cellulose or holocellulose) isolates.

## Materials and methods

### Study site

The Fall River research site is in the Coast Range of Washington State, USA at  $46^{\circ}43'\text{N}$  lat. and  $123^{\circ}25'\text{W}$  long. The climate is maritime with wet, mild winters and warm, relatively dry summers. Mean annual temperature is  $9.2^{\circ}\text{C}$  with monthly means of  $2.6^{\circ}\text{C}$  in the coldest month, and  $16^{\circ}\text{C}$  in the warmest month (Ares et al. 2007b). Estimated mean annual precipitation is 2,260 mm mostly as rain. The experimental site is between 305 and 362 m elevation on gentle slopes ( $<10\%$ ) facing west. The soil is a medial over clayey, ferrihydritic over parasesquic, mesic Typic Fulvudand (Soil Survey Staff 2003) of the Boistfort series developed from weathered Miocene basalt and with some volcanic ash in the upper horizons (Logan 1987). The soil is deep, well-drained, mostly stone-free, and has low bulk density, high organic C content and high water-holding capacity (Ares et al. 2005).

The site was occupied by a Douglas-fir/western hemlock stand which was harvested in 1999. At the time of this study in 2005, the whole experimental site contained seven treatments (Ares et al. 2007b). This investigation deals with two treatments that received conventional forest harvest: (1) complete vegetation control (VC) during 5 years after planting as described in Ares et al. (2007b), and (2) no-vegetation control (NVC). These treatments were chosen because they differed in tree growth rates and soil water content during the growing season. Each treatment was replicated four times in a randomized complete block design. Blocks 1 and 2 were on gentle upper slopes, and blocks 3 and 4 were on gentle lower slopes. Blocks 3 and 4 were immediately below blocks 1 and 2 topographically, but separated by a buffer large enough to accommodate harvesting equipment traffic. Treatment plots are 30 m × 85 m (0.25 ha) with an internal 15 m × 70 m (0.10 ha) measurement area. The buffer area contains three rows of trees. Plant canopy cover of competing vegetation in VC was 5, 2 and 2% in years 3, 4 and 5 after planting, respectively, compared to 83, 92 and 95%, respectively, in NVC (Ares et al. 2007a). All plots were shovel planted with 1 + 1 Douglas-fir seedlings at 2.5-m × 2.5-m spacing (1,600 trees ha<sup>-1</sup>) in March 2000. Twenty additional Douglas-fir seedlings were systematically planted between rows in all plots for biomass sampling at age 5 before crown closure.

An automated weather station was installed in 2000 to provide information on precipitation, air and soil temperature, relative humidity, solar radiation and soil-water content. Precipitation was measured with a 20.3-cm diameter TE5252 tipping bucket rain gauge (Texas Electronics, Dallas, TX, USA).

#### Tree-ring sampling, and $\delta^{13}\text{C}$ and wood density determinations

Within the group of trees that were close ( $\pm 0.5$  cm) in stem diameter at 1.3 m aboveground (DBH) to the arithmetic mean DBH for each plot, we randomly selected eight trees from internal and middle rows of plot buffer areas in VC and NVC plots within each of the four blocks of the study on December 7 and 8, 2004. In total, we selected 64 trees (8 per plot × 2 treatments × 4 blocks). Average DBH of sampled trees was 5.8 cm in VC and 4.4 cm in NVC plots. Height of the selected trees was not measured but average height of VC and NVC trees was 3.6 and 3.1 m, respectively. Each tree stem was cored twice with a 5-mm-diameter increment borer at 30-cm above ground from north- and east-facing stem areas. Wood cores were placed in plastic trays immediately upon collection. Width of annual rings was measured in the laboratory on each core using a ruler with precision and accuracy of 0.5 mm.

Early and latewood of rings for years 3–5 were then separated based on changes in color and resistance to peeling using a steel blade under 10×-magnification of a Leica MZ75 (Leica Microsystems, Wetzlar, Germany) dissecting microscope. Samples were composited per plot by tissue and year, oven dried at 65°C, ground to pass a 1-mm screen size, and analyzed for  $\delta^{13}\text{C}$  at the Stable Isotope Facility of the University of California at Davis by using a continuous-flow mass spectrometer Europa Hydra 20/20 (Europa Scientific Tracermass, Scientific Limited, Crewe, UK). Relative abundance of  $^{13}\text{C}$  and  $^{12}\text{C}$  was expressed as:

$$\delta^{13}\text{C} = [(R_s/R_b) - 1] \times 1,000 \quad (1)$$

where  $R_s$  and  $R_b$  are the  $^{13}\text{C}/^{12}\text{C}$  ratio in the sample and the Pee Dee belemnite standard. Precision of the measurements ranged between 0.06 and 0.10‰ and accuracy determined from blind duplicate samples included to test the repeatability of the C isotope determinations was 0.05‰.

In August 2005, five Douglas-fir trees were sampled from the biomass sample tree population in one VC plot and one NVC plot per block. Branches were removed in the field and intact boles were taken to the laboratory. A 2.5-cm thick disk was obtained at 0.30 m aboveground from each of the five Douglas-fir trees sampled within each of the eight plots. Again, trees that were selected had a DBH similar to the arithmetic mean DBH for each plot, and had normal form and approximately average height. Two out of the five disks per plot were randomly selected for X-ray scanning at Weyerhaeuser Company's Wood Laboratory in Federal Way, WA, USA. If any of the selected disks had the pith too far from the approximate geometric center of the disks, it was discarded for X-ray analysis and another disk was picked. Wood density was determined by X-ray scanning on two radii of wood disks with equilibrium moisture of 12% with a QRTS-01X tree ring scanner (Quintek Measurement Systems, Knoxville, TN, USA). The spatial resolution was 768 pixels per inch. Wood density results are expressed on a dry weight basis. Early and latewood were then separated, oven dried at 65°C, and ground to pass a 1-mm screen size after checking the X-ray density diagram that allowed a better distinction between early and latewood areas, and false rings. The Douglas-fir wood disks analyzed showed a subtle transition between early and latewood, and rings were less distinctive compared with samples from older trees (G. Leaf, personal communication).

#### Crude cellulose and holocellulose extractions

Early and latewood samples separated from the disks were divided into three portions; one remained untreated, the other was treated with acidified di-glycol methyl ether (Diglyme-HCl method) to extract crude cellulose (i.e., pure cellulose and some hemicellulose and lignin) from

wood as described in MacFarlane et al. (1999), and the third portion was processed to holocellulose (i.e., mainly pure cellulose and hemicellulose) by Soxhlet extraction with a 2:1 toluene-ethanol mixture and bleaching with  $\text{NaClO}_2$  (Leavitt and Danzer 1993). The Diglyme-HCl method was applied to 48 samples (2 trees per plot  $\times$  2 blocks  $\times$  2 wood tissues  $\times$  2 treatments  $\times$  3 years). Because of the limited amount available for some samples, the Toluene-ethanol method was only applied to 23 samples. Untreated and treated samples were analyzed for  $\delta^{13}\text{C}$  as described above.

#### Statistical analysis

Vegetation control effects on early and latewood  $\delta^{13}\text{C}$  (both for core and wood-disk sections) were examined as a split-plot mixed-effects model (Littell et al. 1996) of repeated measures data with vegetation control as the main plot fixed effect, year as the subplot fixed effect, and block as a random effect. A repeated measures analysis was appropriate because  $\delta^{13}\text{C}$  measurements were done for different years on the same tree and, therefore, they were not independent. The covariance structure associated with the within-subject factor (i.e., year) was selected based on the Bayesian Criterion and the Akaike's Information criterion. The first-order autoregressive heterogeneous covariance structure provided the lowest values for both criteria. Comparisons of treatment means for each year were made using orthogonal contrasts with one degree of freedom. The relation between  $\delta^{13}\text{C}$  of untreated and treated wood was analyzed by using simple correlation analysis.

## Results

Summer precipitation during 2002 and 2003 (stand ages 3, 4) could be considered typical. During these years, there was little precipitation in July and August (Fig. 1). In 2004 (year 5), summer was generally dry, but substantial precipitation occurred in the last third of August.

Average tree-ring width was greater in trees within plots receiving vegetation control than in plots without vegetation control, in agreement with the trends in stem diameter growth observed in the study (Fig. 2). There were substantial within-ring variations in wood density from a minimum of  $200 \text{ kg m}^{-3}$  in earlywood to a maximum of  $750 \text{ kg m}^{-3}$  in latewood. Average tree density per ring (years 2–5) did not differ between trees in VC and NVC ( $398 \pm 15.3$  and  $414 \pm 11.2$ , respectively).

For the wood-core samples, variability in  $\delta^{13}\text{C}$  values among experimental blocks was low especially for late wood with standard errors ranging from 0.04 to 0.20‰. Earlywood  $\delta^{13}\text{C}$  was not significantly different for trees in

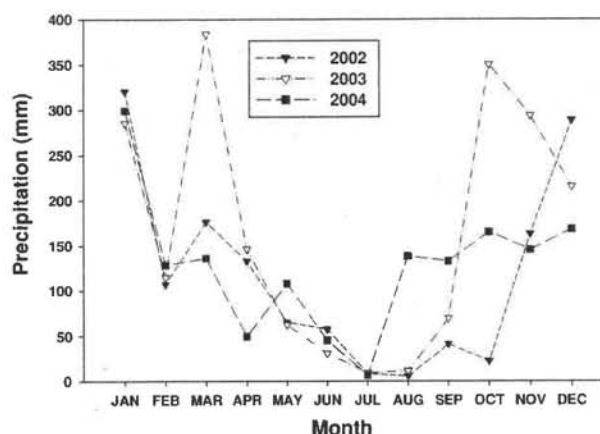


Fig. 1 Mean monthly precipitation recorded from 2002 (plantation age 3) to 2004 (plantation age 5) at Fall River in coastal Washington

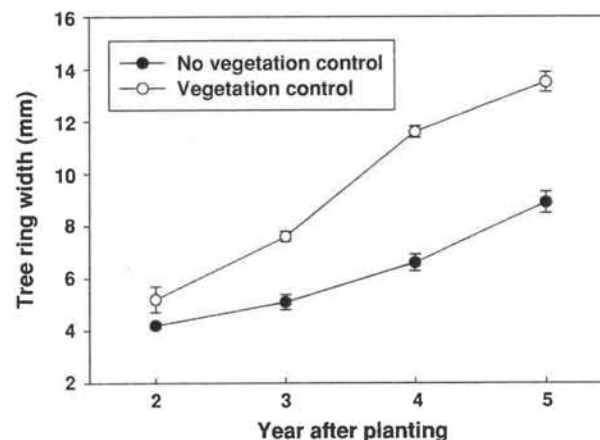


Fig. 2 Tree ring width of selected trees in treatments with and without vegetation control at Fall River in coastal Washington from plantation age 2 (2001) to 5 (2004). Values are mean  $\pm$  one standard error

VC and NVC in any year ( $P = 0.71$ ,  $0.75$  and  $0.12$  for years 3–5) (Table 1). Latewood  $\delta^{13}\text{C}$  was significantly different for trees in VC and NVC in years 4 ( $P = 0.04$ ) and 5 ( $P = 0.02$ ). In these years,  $\delta^{13}\text{C}$  was greater (i.e., less negative) in trees in NVC; this was consistent with the stated hypothesis. Earlywood  $\delta^{13}\text{C}$  differed among years ( $P = 0.02$ ), and the year  $\times$  vegetation control treatment was not significant ( $P = 0.20$ ). Latewood  $\delta^{13}\text{C}$  also differed among years ( $P < 0.01$ ), and the year  $\times$  vegetation control treatment interaction was significant ( $P < 0.001$ ) as expected because there was no difference in  $\delta^{13}\text{C}$  between treatments in year 3.

Earlywood  $\delta^{13}\text{C}$  from wood cores was not significantly different than latewood  $\delta^{13}\text{C}$  in any year ( $P$  from 0.11 to 0.22) in NVC. In VC, earlywood  $\delta^{13}\text{C}$  was greater than latewood  $\delta^{13}\text{C}$  in years 4 and 5 ( $P < 0.001$  and  $P = 0.04$ , respectively).

**Table 1**  $\delta^{13}\text{C}$  (‰) in untreated early and latewood of Douglas-fir trees in plots with and without complete vegetation control at Fall River

Tissue	Vegetation control	Plantation age (year)		
		3 (2002)	4 (2003)	5 (2004)
Earlywood	Y	$-25.8 \pm 0.18$ (-25.4, -26.2) a	$-25.4 \pm 0.03$ (-25.3, -25.5) a	$-26.1 \pm 0.22$ (-25.7, -26.7) a
	N	$-25.9 \pm 0.47$ (-25.1, -26.3) a	$-25.3 \pm 0.28$ (-25.1, -25.7) a	$-25.6 \pm 0.23$ (-25.0, -26.1) a
Latewood	Y	$-25.9 \pm 0.07$ (-25.7, -26.1) A	$-26.3 \pm 0.08$ (-26.1, -26.4) B	$-26.8 \pm 0.04$ (-26.7, -26.9) B
	N	$-26.1 \pm 0.16$ (-25.7, -26.3) A	$-25.5 \pm 0.15$ (-25.2, -25.7) A	$-26.1 \pm 0.17$ (-25.8, -26.3) A

Early and latewood were separated by year from samples taken with an increment borer

Values are mean  $\pm$  one standard error ( $n = 4$ ). Minimum and maximum values are in parentheses. Mean within columns followed by the same letter are not significantly different at  $P \leq 0.05$  for early and latewood analyzed separately

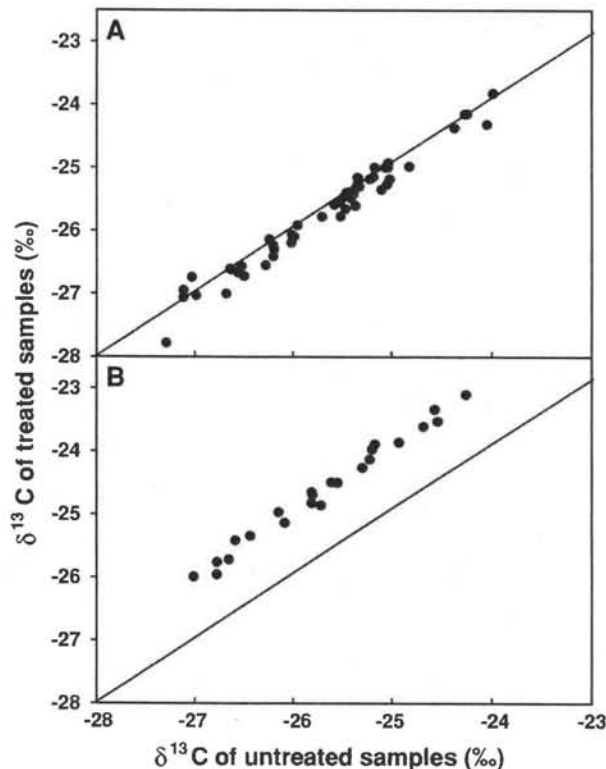
For the wood-disk samples, the variability in  $\delta^{13}\text{C}$  values among experimental blocks was similar to that observed for wood samples obtained by coring. The  $\delta^{13}\text{C}$  values of untreated and Diglyme-HCl treated samples were highly correlated ( $r^2 = 0.97$ ), and almost identical (Fig. 3a).  $\delta^{13}\text{C}$  values of samples treated with the Toluene-ethanol method (i.e., hollocelulose samples) were significantly higher than those of untreated samples by 1–1.2‰ within the  $-24$  to  $-27$ ‰ range of the sample (Fig. 3b); however,  $\delta^{13}\text{C}$  values of treated and untreated samples

were highly correlated ( $r^2 = 0.98$ ). For the two method comparisons, the slopes of the relationship between  $\delta^{13}\text{C}$  of treated and untreated samples were not significantly different from one.

## Discussion

The low variability in  $\delta^{13}\text{C}$  values we observed among blocks and within trees was consistent with the high isotope signal reproducibility often found among and within trees (Leavitt 1993; Dupouey et al. 1993). Intra-annual variations in atmospheric stable isotope composition (i.e., lowest and highest  $\delta^{13}\text{C}$  values in April–May, and June–July, respectively, in the northern hemisphere) should not change the interpretation of the results as effects on wood  $\delta^{13}\text{C}$  should have similarly affected trees in both treatments. Greater  $\delta^{13}\text{C}$  (i.e., decreased  $^{13}\text{C}$  discrimination) in latewood of Douglas-fir at age 4 and 5 in NVC compared to VC supports the contention that soil water supply and/or water uptake for Douglas-fir trees was less in NVC than in VC during the second half of these growing seasons. This might indicate greater photosynthesis:stomatal conductance ratios leading to increased intrinsic water-use efficiency among trees in NVC compared to those in VC plots. The lack of differences in  $\delta^{13}\text{C}$  in earlywood of trees in the two vegetation control treatments may reflect no differences in water-use efficiency between treatments during the early part of the growing season when precipitation is high and evaporative demand is low (Devine and Harrington 2006). At Fall River, mean foliage  $\delta^{13}\text{C}$  was identical (i.e.,  $-29.2$ ‰) in 3-year-old Douglas-fir in treatments with and without vegetation control (A. Ares, personal observation). These results suggested that foliage  $\delta^{13}\text{C}$  did not differ as  $\delta^{13}\text{C}$  primarily represented structural C fixed during the initial part of the growing season (e.g., May and June) when there was ample soil water supply for both trees and understory vegetation.

Differences in latewood  $\delta^{13}\text{C}$  in years 4 and 5 support the hypothesis that Douglas-fir growth was depressed by reduced soil water availability in NVC. In August of year 4,



**Fig. 3** Relationships between  $\delta^{13}\text{C}$  of untreated ( $\sigma^{13}\text{C}_u$ ) Douglas-fir wood samples and samples processed with the Diglyme-HCl ( $\sigma^{13}\text{C}_d$ ) (a) or the Toluene-ethanol ( $\sigma^{13}\text{C}_e$ ) (b) methods. For a,  $\sigma^{13}\text{C}_d$  (‰) =  $0.69 + 1.03 \sigma^{13}\text{C}_u$  (‰),  $r^2 = 0.97$ ,  $n = 50$ ; for b,  $\sigma^{13}\text{C}_e$  (‰) =  $2.61 + 1.06 \sigma^{13}\text{C}_u$  (‰),  $r^2 = 0.98$ ,  $n = 23$ . Diagonal lines indicate a 1:1 relationship

soil water potential at 30-cm soil depth ranged between  $-348$  and  $-290$  kPa in VC compared to  $-126$  to  $-235$  kPa in NVC (Devine and Harrington 2006). Stem diameter growth increment at year 4 was positively correlated with soil water content ( $P < 0.001$ ) at 30-cm soil depth. Soil water potentials were less negative in the early growing season and differences between treatments during this period were generally not significant. In years 4 and 5, shading of the Douglas-fir tree crowns by understory (mostly herbaceous) vegetation in NVC was minimal; thus, it is unlikely that changes in wood  $\delta^{13}\text{C}$  was caused by variations in the light environment under which the trees grew.

Significantly greater  $\delta^{13}\text{C}$  in earlywood than in latewood of trees in years 4 and 5 on plots where vegetation was controlled was unexpected although similar results were reported for young *Picea abies* L. (Karst) trees (Jäggi et al. 2002).  $\delta^{13}\text{C}$  of earlywood may be more dependent on C reserves (Schulze et al. 2006), and related to biochemical fractionation (e.g., during starch formation) than to environmental effects (Jäggi et al. 2002). In hardwood species, 10–20% of late wood can be built by remobilized products so storage process can affect its  $\delta^{13}\text{C}$  composition (Helle and Schleser 2004; Skomarkova et al. 2006) but this effect has not been studied in Douglas-fir. Also, it has been proposed that a significant proportion of root C, which is isotopically heavier than stem C could be incorporated into earlywood (Livingston and Spittlehouse 1996).

Untreated and cellulose-isolated wood samples were equally useful in this study for comparing vegetation control effects on  $\delta^{13}\text{C}$  patterns in young Douglas-fir. Whole-tissue and cellulose  $\delta^{13}\text{C}$  values often have mid to very high correlations for leaves (e.g.,  $r^2 = 0.92$ – $0.97$  in ponderosa pine, Gomez et al. 2002) and wood (e.g.,  $r^2 = 0.79$  in 20-year-old Douglas-fir, Livingston and Spittlehouse 1996;  $r^2 = 0.84$  for tropical tree species, West et al. 2001;  $r^2 = 0.92$  in *Eucalyptus globulus* Labill., *Pinus radiata* D. Don., and *Pinus pinaster* Ait., MacFarlane et al. 1999;  $r^2 = 0.99$  in *Pinus contorta* Dougl. ex Loud., Guy and Holowachuk 2001). At Fall River,  $\delta^{13}\text{C}$  of untreated wood samples and cellulose isolates were very closely correlated.

The one-step Diglyme–HCl method has been recommended to extract crude cellulose because it is fast and does not require special glassware (MacFarlane et al. 1999). In that study, average  $\delta^{13}\text{C}$  of crude cellulose samples of *Eucalyptus globulus*, *Pinus radiata*, and *Pinus pinaster* extracted with the Diglyme–HCl method was about 0.8‰ higher than that of untreated wood compared to a difference of 0.3‰ in this study. Fall River samples after the Diglyme–HCl step were brown and may have contained residual lignin; this possibility is suggested by results reported by MacFarlane et al. (1999) where only about 40% of the cellulose was extracted. A follow-up

bleaching stage has been proposed for the Dyglime–HCl method (Cullen and MacFarlane 2005) but it was not tested in this study. Douglas-fir has relatively high yield of extractives (i.e., resin, lipids, organic acids, terpenes, phenols and waxes) and a medium yield of holocellulose (52% from extractive-free wood in Harlow et al. 2006).

## Conclusions

Separating early and latewood within tree rings of young Douglas-fir grown in an area with seasonally dry summers was useful to more closely examine the effects of reduced water supply on  $\delta^{13}\text{C}$ . This may be most relevant when treatments may differ in their effects during different portions of the cambial growing season (i.e., earlywood vs. latewood). For example,  $\delta^{13}\text{C}$  for trees without vegetation control was greater in latewood formed during the later part of the growing season when competition for water between understory vegetation and trees is more intense, and evapotranspiration: precipitation ratios increase. This provided additional evidence that tree growth reduction in NVC at the Fall River study site in Coastal Washington was mediated by reduced water supply as there was little shading from the understory vegetation at this stage in plantation development.

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