ECOPHYSIOLOGY

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Environmental sensitivity of gas exchange in different-sized trees

Abstract The carbon isotope signature ($\delta^{13}C$) of foliar cellulose from sunlit tops of trees typically becomes enriched as trees of the same species in similar environments grow taller, indicative of size-related changes in leaf gas exchange. However, direct measurements of gas exchange in common environmental conditions do not always reveal size-related differences, even when there is a distinct size-related trend in $\delta^{13}C$ of the very foliage used for the gas exchange measurements. Since $\delta^{13}C$ of foliage predominantly reflects gas exchange during spring when carbon is incorporated into leaf cellulose, this implies that gas exchange differences in different-sized trees are most likely to occur in favorable environmental conditions during spring. If gas exchange differs with tree size during wet but not dry conditions, then this further implies that environmental sensitivity of leaf gas exchange varies as a function of tree size. These implications are consistent with theoretical relationships among height, hydraulic conductance and gas exchange. We investigated the environmental sensitivity of gas exchange in different-sized Douglas-fir (*Pseudotsuga menziesii*) via a detailed process model that specifically incorporates size-related hydraulic conductance [soil–plant–atmosphere (*SPA*)], and empirical measurements from both wet and dry periods. *SPA* predicted, and the empirical measurements verified, that differences in gas exchange associated with tree size are greatest in wet and mild environmental conditions and minimal during drought. The results support the hypothesis that

annual net carbon assimilation and transpiration of trees are limited by hydraulic capacity as tree size increases, even though at particular points in time there may be no difference in gas exchange between different-sized trees. Maximum net ecosystem exchange occurs in spring in Pacific Northwest forests; therefore, the presence of hydraulic limitations during this period may play a large role in carbon uptake differences with stand-age. The results also imply that the impacts of climate change on the growth and physiology of forest trees will vary depending on the age and size of the forest.

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Introduction

The Hydraulic Limitation Hypothesis proposed that the ultimate limit to height growth and primary production of trees was due to size-induced constraints on water transport (Ryan and Yoder 1997). The hypothesis is based on the tenet that as trees grow in height, the supply of water to foliage is reduced due to a reduction in leaf-specific hydraulic conductance (k_1). This reduction in k_1 occurs due to increased friction and gravitational restrictions unless other components of the hydraulic system, such as the ratio between leaf area and water conducting area in the sapwood or the size of water-conducting cells, change in compensation. In homeohydric plants, those which regulate stomatal conductance to prevent leaf water potential from falling below a specific minimum, a reduction in k_1 typically results in reduced time-averaged stomatal conductance (g_s, Sperry et al. 1993; Hubbard et al. 2001), thereby limiting transpirational water loss but simultaneously limiting the diffusion of CO_2 into the sites of carboxylation within foliage. This reduction in CO_2 diffusion

reduces the CO_2 concentration inside the leaf (c_i), subsequently reducing photosynthetic assimilation (A) and growth (Gower et al. 1996; Ryan et al. 1997). The Hydraulic Limitation Hypothesis has been examined in numerous field studies (Yoder et al. 1994; Mencuccini and Grace 1996a, b; Hubbard et al. 1999; Schäfer et al. 2000; Kolb and Stone 2000; Ryan et al. 2000; Phillips et al. 2002; McDowell et al. 2002a; Barnard et al. 2003), modeling studies (Murty et al. 1996; Magnani et al. 2000; Williams et al. 2001a) and through other assessments (Becker et al. 2000; Bond et al. 2000; Mencuccini and Magnani 2000; Midgley 2003; Mencuccini 2003; Ryan et al. 2004).

One approach to assessing hydraulic limitations is through measurement of foliar gas exchange in trees with naturally varying or experimentally altered k_l. Direct measurements can be accomplished using infrared gas analyzers with leaf-scale cuvettes for measurement of A and g_s. Hydraulic limitations are supported if lower values of A and g_s are associated with lower values of k_l. Another approach utilizes the stable carbon isotope ratio ($\delta^{13}C$) of foliage tissue because $\delta^{13}C$ is related to gas exchange via the following simplified equations (Farquhar et al. 1989):

$$\delta^{13}C \approx \delta^{13}C_a - a - (b - a) \cdot \frac{c_i}{c_a}, \tag{1}$$

$$c_i \approx c_a - \frac{A}{g_s}, \tag{2}$$

where $\delta^{13}C_a$ is the isotopic composition of atmospheric CO_2, a is the fractionation associated with diffusion in air (4.4‰), b is the net fractionation associated with carboxylation by Rubisco (27–29‰), and c_i and c_a are the intercellular and atmospheric partial pressures of CO_2, respectively. Carbon isotope discrimination (Δ) can be calculated approximately by moving $\delta^{13}C_a$ to the left hand side of Eq. 1, and represents the net physical, chemical and biological effect of discrimination processes. From Eqs. 1 and 2 we can see that $\delta^{13}C$ is proportional to the ratio of A to g_s via shifts in c_i (Ehleringer et al. 1993). Typically, a decrease in g_s causes an increase in $\delta^{13}C$ (Ehleringer 1993, Meinzer et al. 1993) and in water-limited conditions, g_s tends to override A in regulating $\delta^{13}C$ (Panek and Waring 1997). Accordingly, we predict that if hydraulic constraints to gas exchange increase with tree size, then Δ will decrease and $\delta^{13}C$ will become more positive with increasing size. Increasing foliar $\delta^{13}C$ with increasing tree size for individuals of the same species grown in similar environments has been observed in numerous studies (Schoettle 1994; Yoder et al. 1994; Hubbard et al. 1999; McDowell et al. 2002a; Phillips et al. 2003; Koch et al. 2004).

We have indirect evidence that size-related variation in c_i and subsequent $\delta^{13}C$ of leaf photosynthate may vary seasonally. In a Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*) chronosequence study, foliar cellulose $\delta^{13}C$ collected from near the crown tops exhibited significant enrichment from -26.1‰ for 15 m tall trees to -23.6‰ for 60 m tall trees, consistent with the hydraulic limitation hypothesis (McDowell et al. 2002a). However, we did not detect size-related differences in A and g_s from cuvette-based measurements of gas exchange in that study. We speculated that the differences in results between the two methods were due to seasonal differences in gas exchange. Foliar cellulose $\delta^{13}C$ represents an assimilation-weighted record of gas exchange (Brugnoli et al. 1998) that occurred in the spring months prior to and during leaf elongation (May through early June). The climate of this region is characterized by wet conditions in April through June, and dry conditions from July through September. Therefore, we hypothesized that hydraulic constraints to gas exchange are manifest during the spring, when atmospheric and soil water content is high, and that such gas exchange limitations are much less predominant during drought.

Theoretical evidence for the hypothesis that hydraulic limitations are manifested primarily during relatively wet periods can be seen by examination of a simplified hydraulic model (Whitehead et al. 1984, Whitehead 1998). The model is a hydraulic corollary to Darcy's Law applied to trees:

$$g_s = \frac{k_l(\Psi_s - \Psi_l)}{D}, \tag{3}$$

in which k_l is hydraulic conductance, Ψ_s is soil water potential, Ψ_l is daytime leaf water potential, and D is vapor pressure deficit (kPa). See Whitehead et al. (1984), Whitehead and Hinckley (1991), Whitehead (1998), Oren et al. (1999), Schäfer et al. (2000), Phillips et al. (2002) and McDowell et al. (2002a) for more complex versions of the model. Using this simplified model, we can make generalized predictions of how g_s, and hence $\delta^{13}C$ (Eqs. 1, 2) may respond to changes in D or Ψ_s at different values of k_l. When k_l is high (e.g., in young and short trees) g_s is more sensitive to D or Ψ_s, i.e., it has a steeper slope than when k_l is low (e.g., in old and tall trees, Fig. 1a, b). As a result, g_s is expected to be most different between trees of different sizes in moist conditions, but in drought conditions, predicted g_s values converge regardless of k_l. Consistent with this prediction, Oren et al. (1999) showed that stomatal sensitivity to D was positively correlated with maximum g_s such that trees with high g_s at low D (e.g., trees with high k_l) were more sensitive to drought than trees with low g_s (e.g., trees with low k_l). Other factors such as changes in the ratio of leaf area to sapwood area (e.g., Meinzer et al. 2001, McDowell et al. 2002b) may cause variations in the model predictions; however, these general predictions serve as useful hypotheses.

The objective of this study was to determine whether environmental sensitivity of gas exchange in coniferous trees is dependent on tree size. We tested this hypothesis through two approaches using Douglas-fir trees. First, we measured foliar gas exchange in tall versus short trees in spring (May) for comparison to previous measure-
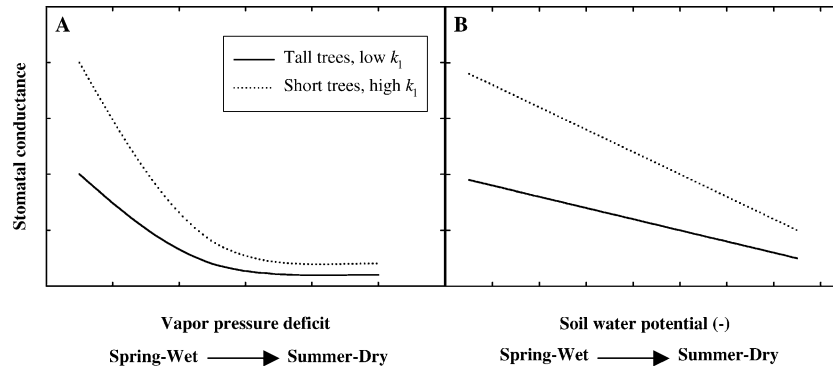
Fig. 1 Predictions of g_s using Eq. 3. In both figures k_l was set to values of one and two to represent short versus tall trees. **a** The response of g_s to increasing D, **b** the response of g_s to Ψ_s. Because in the Pacific Northwest D increases and Ψ_s becomes more negative as the seasons progress from spring to summer, the x-axes are arranged in that direction

ments done during the summer drought (July–September). Second, we modeled gas exchange and subsequent daily, assimilation-weighted $\delta^{13}C$ of foliar photosynthate for the two size classes of Douglas-fir using an ecosystem process model, (soil–plant–atmosphere (*SPA*), Williams et al. 1996) parameterized for these forests. We reasoned that if our hypothesis was correct, both direct and modeled measurements would show large size-related differences in gas exchange in spring and diminished differences in summer.

Methods

A point on nomenclature

Terms related to age and size of trees are sometimes confused in the Hydraulic Limitation literature. In this manuscript, the terms "young" and "old" trees are not used to infer that age, rather than size, is the driving factor for differences among stands. Direct tests of the role of age versus size are currently underway (B. Bond, unpublished data, M. Mencuccini, in review). The terms "height" and "size" are also sometimes confused. In this manuscript, we often refer to changes in gas exchange with tree size rather than height because we are referring explicitly to the tops of trees that are of different size, rather than changes in gas exchange across the height continuum within a crown of individual trees.

Site description

Our study sites were two stands of Douglas-fir located within the Wind River basin of the Cascade Mountains near Carson, Washington (45°49′N Latitude, 121°57′ W Longitude). The climate of this region is maritime, with cool, wet winters and warm, dry summers. Annual precipitation is approximately 2,500 mm, with less than 10% of that falling between June and September (~120 mm). The soils are loamy sands and sandy loams

developing over 2–3 m of volcanic tephra. The two stands are within 10 km and 200-m elevation of each other and are described in detail in Phillips et al. (2002). The average height and age were 17 and 60 m and 22 and 450 years, respectively. Leaf area index was ~ 8.6 m^2 m^{-2} ground area for both the 22 and 450 year old stands (Thomas and Winner 2000, Parker et al. 2004), and stand density is ~21,250 and 448 stems ha^{-1}, respectively. Leader height growth is ~0.96 and 0.06 m $year^{-1}$, respectively (McDowell et al. 2002a). The 22-year-old stand originated after a clearcut, whereas the 450-year-old originated after a stand-replacing fire (Franklin and DeBell 1988). A canopy access tower was erected in the center of the young stand, and a construction crane was used for crown access in the old stand. Branches from five trees were selected that appeared relatively typical of each stand and showed no visible damage. Foliage gas exchange was measured from three branches per tree in each stand.

Gas exchange measurements

Diurnal foliar gas exchange was measured at each stand on two consecutive days in May 2002 using identical protocol as in July–September 1999 (McDowell et al. 2002a). Using two Li-Cor 6400 portable photosynthesis systems (Li-Cor, Lincoln, NE, USA), two teams of two people measured gas exchange in the young and old stands simultaneously on day one (May 1), and then the two teams switched stands and repeated the simultaneous measurements again the following day (May 2). The day prior to the first day of measurements we compared g_s and A from the two instruments on a seedling and observed no differences between instruments (paired t test, $P = 0.78$, $n = 10$). Both instruments were equipped with a blue-red artificial light source, and irradiance was set at 1,400 μmol photons m^{-2} s^{-1} ; photosynthesis saturates by 1,000 μmol photons m^{-2} s^{-1} in the upper crown of Douglas-fir (Lewis et al. 2000). Cuvette [CO_2] was set to 40 Pa. Hourly mea-

surements were made using 1-year old foliage within the top 10% of the live crown and from all aspects of the trees from ˜7:00 am until late-afternoon. One shoot was repeatedly measured per branch per day, and during each time period, measurements from all three branches per tree were averaged. It was slightly overcast and cool on May 1 and cloudy and cool on May 2, but no rain fell during either day. Average photosynthetic flux density and vapor pressure deficit during the daylight hours on May 1 and May 2 were 625 and 275 $\mu mol\ m^{-2}\ s^{-1}$ and 0.44 and 0.26 kPa, respectively.
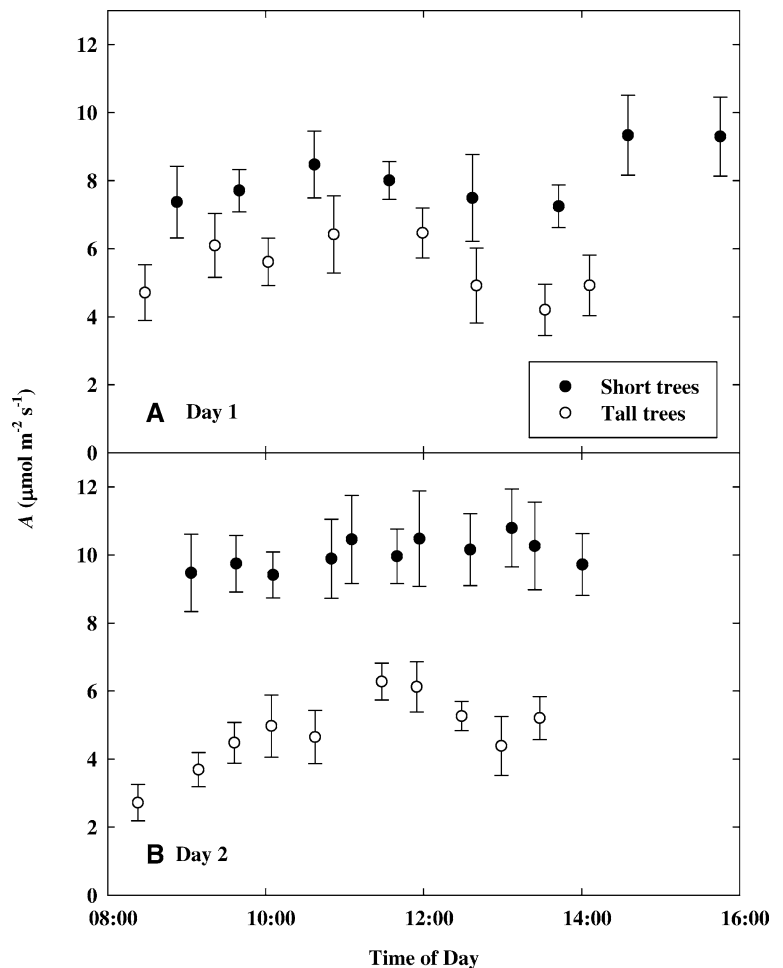
Model analyses

The *SPA* model is a 30-min time-step, processes-based model, where the structure of the canopy is divided into 10 layers to represent the vertical variation of the physical environment and leaf biochemical features (Williams et al. 1996; Law et al. 2000). The soil-roots submodel has 20 layers, allowing a detailed vertical representation of root distribution and water dynamics as a function of soil depth (Williams et al. 2001a). *SPA* uses a pipe hydraulic scheme approach analogous to an electric circuit, assuming that each canopy layer is served by an independent water supply system of roots. Leaf water potential varies with transpiration, which is calculated by the Penman-Montieth equation.

Photosynthesis and transpiration are linked through g_s, which is controlled to maximize carbon gain per unit nitrogen within the limits set by the rate of water uptake and canopy water storage. The model accounts for many structural and physiological differences in the stands, including height, stem and root resistance, leaf area and root vertical distribution, minimum mid-day leaf water potential, canopy layer capacitance, rooting depth, and photosynthetic capacity. We added a sensitivity analysis of the effects of variable mesophyll conductance on Δ (see Results). The model output appeared reliable when compared to empirical sapflow measurements from day 90 to 300, 2002. Regression results of predicted versus observed half-hourly measurements of sapflow density for this period gave r^2 and slopes of 0.82 and 1.004, respectively, for the young stand, and 0.79 and 0.973, respectively, for the old stand. More information on the current model status and validation can be found in Licata (2003).

The stable carbon isotope ratio of photosynthate, i.e., the assimilation weighted products of photosynthesis, was calculated for each daily period using the equation:

Fig. 2 Photosynthesis measurements collected from the 17 m and 60 m tall stands on 1 May 2002 (**a**), and 2 May 2002 (**b**). Five trees with three branches each were measured and averaged for each point. Bars are standard errors

$$\delta^{13}C = -12.4 - \left(22.6 \cdot \frac{\tau c_i}{355}\right), \tag{4}$$

where τc_i is the daily time averaged c_i per canopy layer, weighted by net assimilation rate at each timestep. τc_i is calculated using Eq. 5 where A_i is the net assimilation rate per timestep.

$$\tau c_i = \frac{\sum A_i \cdot c_{ii}}{\sum A_i}. \tag{5}$$

Statistical analyses

Repeated measures analysis of variance was used to test for differences associated with tree size for cuvette measurements of A, g_s, and c_i/c_a. SYSTAT 10.0 was used for analysis.

Results

Gas exchange parameters of Douglas-fir trees differed for the 17 m and 60 m trees on May 1 and 2, 2002. On both days, A was higher for the short trees than the tall

trees (Fig. 2) and g_s was higher for the short trees than the tall trees (Fig. 3). The ratio c_i/c_a was higher for short trees than tall trees on both dates as well (Fig. 4). Differences in gas exchange were significant at $\alpha = 0.01$ for A, g_s and c_i/c_a. For comparison to our previous gas exchange results in which no statistical difference was observed between tree size classes, we have plotted the current (2002) g_s data with the 1999 g_s data from McDowell et al. (2002a) in Fig. 5.

Model estimates of Δ (shown in Fig. 6a as photosynthate $\delta^{13}C$) exhibited greater Δ (more negative $\delta^{13}C$) for short than for tall trees (Fig. 6a). To test the hypothesis that differences in Δ between short and tall trees are greater in the spring than summer, we calculated the difference between the tall versus short photosynthate $\delta^{13}C$ shown in Fig. 6a. This difference estimate is shown in Fig. 6b. Tall trees were up to 2.0‰ more enriched than short trees on day 150 and then became progressively more similar, reaching a $\delta^{13}C$ difference of 0.0‰ on day 250.

The model predictions in Fig. 6 used nonlimiting values of mesophyll conductance (g_i, the leaf internal conductance of CO_2 from the substomatal pore to the site of carboxylation within the chloroplast [Evans et al. 1986]). In reality, however, g_i is not infinite, and differ-

Fig. 3 Stomatal conductance measurements collected from the 17 m and 60 m tall stands on 1 May 2002 (a), and 2 May 2002 (b). Five trees with three branches each were measured and averaged for each point. Bars are standard errors
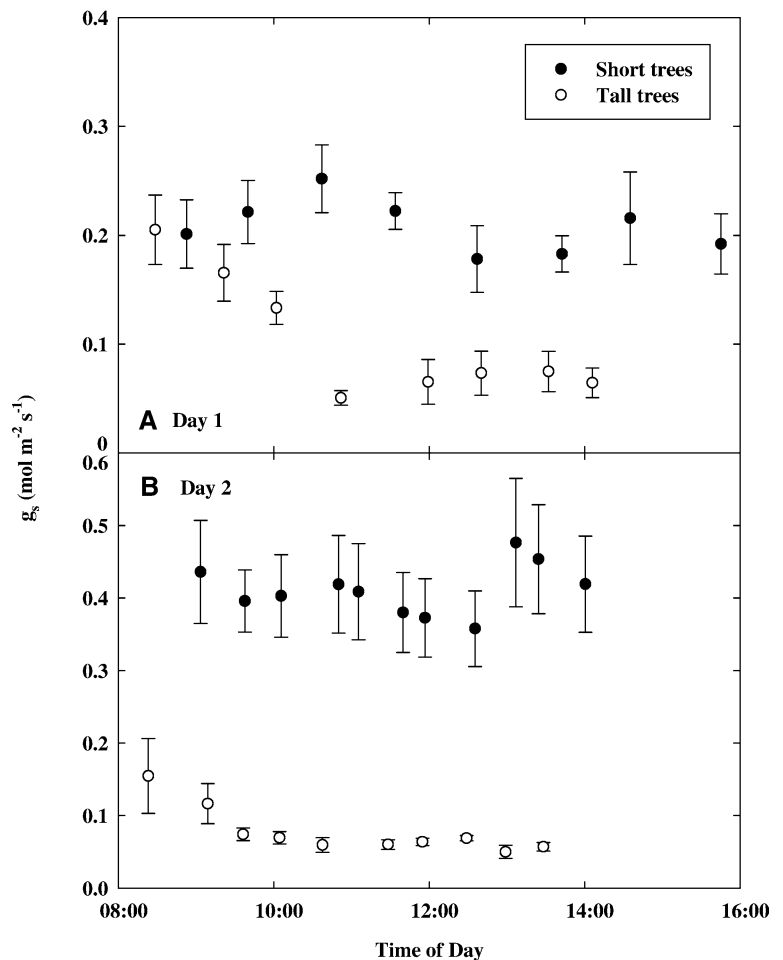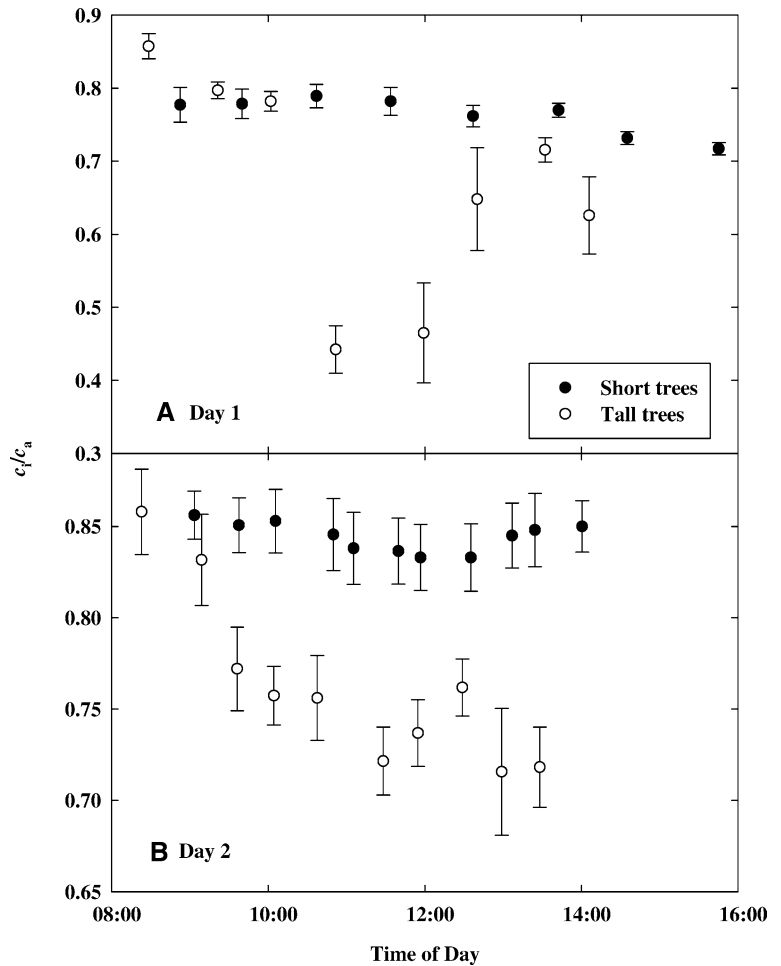
Fig. 4 Measurements of c_i/c_a collected from the 17 m and 60 m tall stands on 1 May 2002 (**a**), and 2 May 2002 (**b**). Five trees with three branches each were measured and averaged for each point. Bars are standard errors

Fig. 5 Stomatal conductance measurements collected from the 17 m and 60 m tall stands in May 2002 and July, August, and September 1999 (from McDowell et al. 2002a). Five trees with three branches each were measured and averaged for each point. Bars are standard deviations
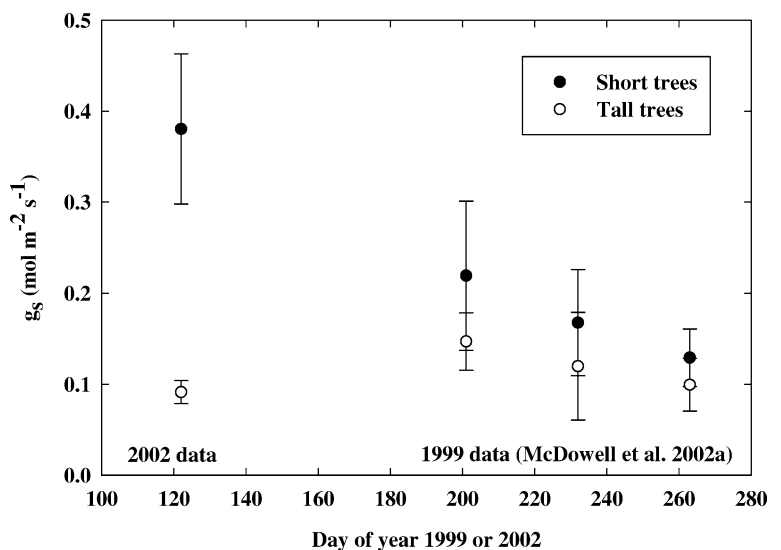
ences in g_i can affect Δ. We have no direct measurements of g_i, but it is reasonable to suspect that g_i is greater in leaves of short than tall trees because their mass per unit leaf area and leaf thickness tends to be lower, and thus the pathlength for diffusion of CO_2 is shorter. We conducted a sensitivity analysis of *SPA*'s $\delta^{13}C$ predictions over a range of g_i values (0.05–0.2 mol m^{-2} s^{-1}) to determine whether our conclusions are robust regardless

Fig. 6 a Model predictions of foliar photosynthate $\delta^{13}C$ for the 17 m and 60 m tall stands for day 100 through 300, 2002. **b** The $\delta^{13}C$ difference between tall and short trees in (**a**) calculated as tall $\delta^{13}C$ minus short $\delta^{13}C$. The smoothed line is the weekly average $\delta^{13}C$ difference, shown to highlight the seasonal trend. The decline from mid to late summer supports the hypothesis that hydraulic limitations to gas exchange are manifest more strongly during the moist spring season than during drought, or in other words, carbon isotope discrimination of short trees is more sensitive to drought than tall trees
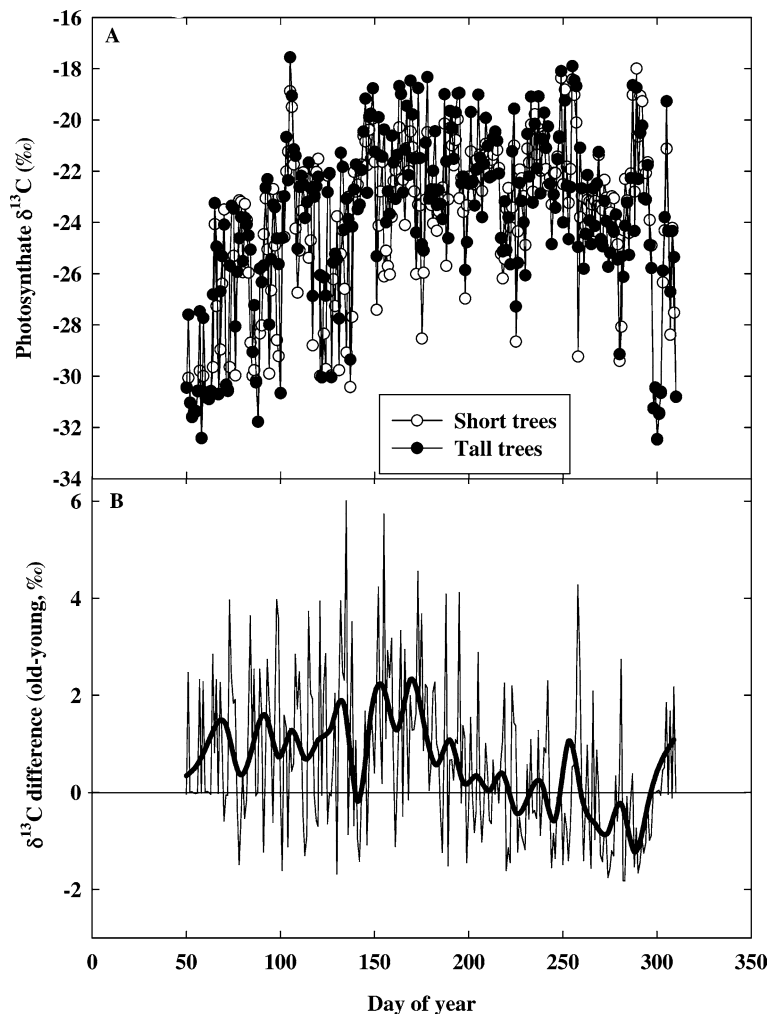
of errors in this parameter. The upper end of the range came from a g_i study on Douglas-fir (50-year-old trees, Warren et al. 2003) and the lower end from Hanba et al. (1999). A representative subset of the model runs are shown in Fig. 7. Regardless of the assumed g_i values, *SPA* predictions of the $\delta^{13}C$ difference between short and tall trees remained similar to the predicted values in Fig. 6b and similar to the gas exchange data shown in Figs. 2, 3, 4 (Fig. 7).

We used *SPA* to investigate size-dependent sensitivity of Δ to the environmental variables identified in Fig. 1. We began with an examination of the hypotheses shown in Fig. 1a, using daily foliar photosynthate $\delta^{13}C$ as our response metric rather than g_s. Figure 8a shows *SPA* predictions of $\delta^{13}C$ in response to increasing D. At D below 0.5 kPa, $\delta^{13}C$ was significantly more positive for the short than the tall stand (ANCOVA, $P < 0.01$), but there was no significant difference between age classes at D greater than 0.5 kPa ($P = 0.19$). The $\delta^{13}C$ difference between short and tall trees, calculated from the data in Fig. 8a, shows a similar pattern to that in Fig. 6b, with the short trees having much higher discrimination (lower $\delta^{13}C$) than the tall trees at low D, with the difference

diminishing at higher D (Fig. 8b). D itself did not vary between the two sites ($p = 0.67$).

We tested the hypothesis shown in Fig. 1b using *SPA* predictions of photosynthate $\delta^{13}C$ of the short and tall trees in relation to soil water content, which is correlated with Ψ_s. At soil water content values above 22%, such as occurs in the spring in this region, the tall trees had significantly higher $\delta^{13}C$ than the short trees ($P = 0.01$), but there was no significant difference below SWC of 22% ($P = 0.49$, Fig. 9).

Discussion

The hypothesis that size-related differences in Douglas-fir gas exchange are a function of atmospheric and soil water content (Fig. 1a, b) was supported through both empirical and modeled results. Rates of Douglas-fir gas exchange in May 2002 (Figs. 2, 3) were substantially higher for short, young trees than tall, old trees, which is consistent with the Hydraulic Limitation Hypothesis (Ryan and Yoder 1997). These large gas exchange differences during wet and mild climatic conditions
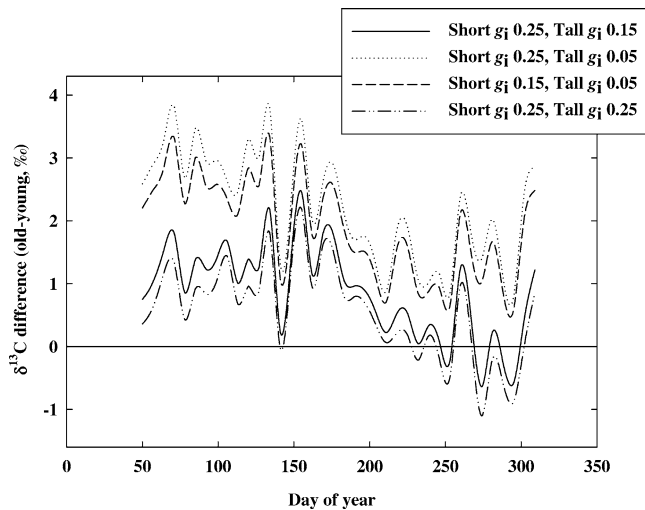
Fig. 7 Model analysis of the sensitivity of $\delta^{13}C$ difference predictions (e.g., Fig. 5) to different assumed values of mesophyll conductance. Mesophyll conductance was varied from the original values (0.25 mol m^{-2} s^{-1} for short and tall trees, respectively) to 0.25 versus 0.15 mol m^{-2} s^{-1}, 0.25 versus 0.05 mol m^{-2} s^{-1}, and 0.15 versus 0.05 mol m^{-2} s^{-1} for the short and tall trees, respectively. This sensitivity analysis indicates that regardless of the assumed values for mesophyll conductance, the $\delta^{13}C$ difference always declines as the year progresses

contrast strongly with the observations during the hot and dry summer months, which were statistically indistinguishable across tree size classes (Fig. 5, McDowell et al. 2002a). Additionally, c_i/c_a ratios in the spring months (Fig. 4) are consistent with the foliar $\delta^{13}C$ data from McDowell et al. (2002a) that showed significant enrichment with increasing tree size. Finally, *SPA* predicted differences in $\delta^{13}C$ between tree size classes (Figs. 6, 7, 8, 9) that are consistent with the measurements of foliar cellulose $\delta^{13}C$ from McDowell et al. (2002a) and with predictions from Fig. 1a and b. Taken together, all evidence suggests that size-related hydraulic limitation to gas exchange is manifest in relatively cool, moist conditions much more than in hot, dry conditions.

The original predictions from the Darcy's corollary hydraulic model (Fig. 1) were qualitatively consistent with *SPA* predictions and our empirical observations. We note that the hydraulic model used by us (Eq. 3) examined changes only in D, Ψ_s, and k_l despite our knowledge that other variables compensate for tree size effects on g_s in Douglas-fir, such as leaf area to sapwood area ratio, mid-day leaf water potential, sapwood porosity, and capacitance (McDowell et al. 2002a, 2002b, Phillips et al. 2002, 2003). However, inclusion of these factors in a more complex version of the hydraulic model showed that compensatory changes do not fully compensate for height-related constraints on k_l; they only reduce the magnitude of the decline in conductance with height (McDowell et al. 2002a). These results from the more complex model derivation shown in McDowell et al. (2002a) coupled with the consistent decline in gas exchange with tree size shown in this study (Figs. 2, 3, 4,

5) support the contention that the prediction of a decline in conductance with increasing tree size will be maintained regardless of model complexity. More important to this study, the corroboration between the simple hydraulic model's predictions, *SPA*'s predictions, and the empirical gas exchange measurements suggests that a more complex hydraulic model is not needed to capture the pattern of environmental dependence of size-dependent variation in Δ.

However, by using a complex process model to study the seasonal dependence of size related gas exchange, we can generate testable hypotheses regarding mechanisms underlying our observations. An important assumption of *SPA* is that aboveground hydraulic resistance (the inverse of k_l) increases with tree height (Williams et al. 1996). As soil water content declines with the progression of summer drought, *SPA* predicts that the fraction of aboveground resistance to total hydraulic resistance remains high in tall trees, however, this fraction declines in short trees (data not shown). In other words, the belowground resistance becomes more important in limiting water use in short trees during drought (e.g., Sperry et al. 1998, 2002; Hacke et al. 2000), whereas aboveground resistance remains a primary constraint on transpiration in tall trees regardless of soil water content. This size-dependent response of belowground conductance to environmental conditions may be compounded if taller trees have more developed root systems that access groundwater (Dawson 1996), which can result in greater hydraulic redistribution and less root embolism (Domec et al. 2004) and reduced seasonal variability of carbon isotope discrimination in the taller trees (McDowell et al. 2004). Additionally, *SPA* predicts that soil water depletion may be greater in young stands due to higher initial transpiration rates (J. Licata and B.J. Bond, unpublished data, and see *x*-axis in Fig. 8). That belowground resistance is more important in short trees than tall trees is a logical expectation; trees that have reached their maximum heights have also reached the minimum sustainable k_l as defined by tree structure and local long-term (decadal to millennial) environment (Mencuccini 2003). This low k_l is relatively dominated by pathlength, which is seasonally invariant. Therefore, we predict that size-dependent environmental sensitivity of gas exchange is partially a result of differential allocation of total system hydraulic resistance to above-versus belowground components.

The *SPA* model includes numerous assumptions that have been thoroughly tested in coniferous forests of the Pacific Northwest (Williams et al. 2001a, b; Licata 2003; Winner et al. 2004). Here, we will briefly address a few assumptions that are novel to this study or that have not been examined previously. Our modeling tests of the hypotheses embodied by Figs. 1a and b (Figs. 8, 9) were conducted using the simplifying assumption that g_s and Δ are linearly correlated. However, this may be an invalid assumption when examined across seasons due to changes in leaf physiology with drought and age. Photosynthetic capacity can decline seasonally

Fig. 8 Model predictions of foliar photosynthate $\delta^{13}C$ as a function of daylight average D (calculated as 2/3 maximum D). Regressions of the response of $\delta^{13}C$ to D from 0 to 0.5 kPa D are significantly different between the short and tall trees, with the short trees having higher $\delta^{13}C$ than the tall trees ($p < 0.01$). Above 0.5 kPa D, there is no difference between the short and tall trees ($P = 0.19$). This is highlighted in Fig. 7b, in which the $\delta^{13}C$ difference between tall and short trees is shown. The $\delta^{13}C$ difference is calculated as in Fig. 5 but using the data from Fig. 7a. The thicker line represents a 10-point moving average and is shown to highlight the overall trend. This analysis supports the hypothesis encapsulated by Eq. 3 and Fig. 1a
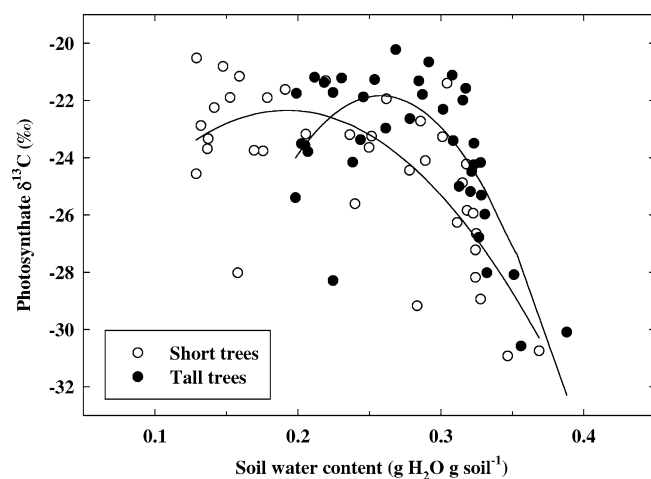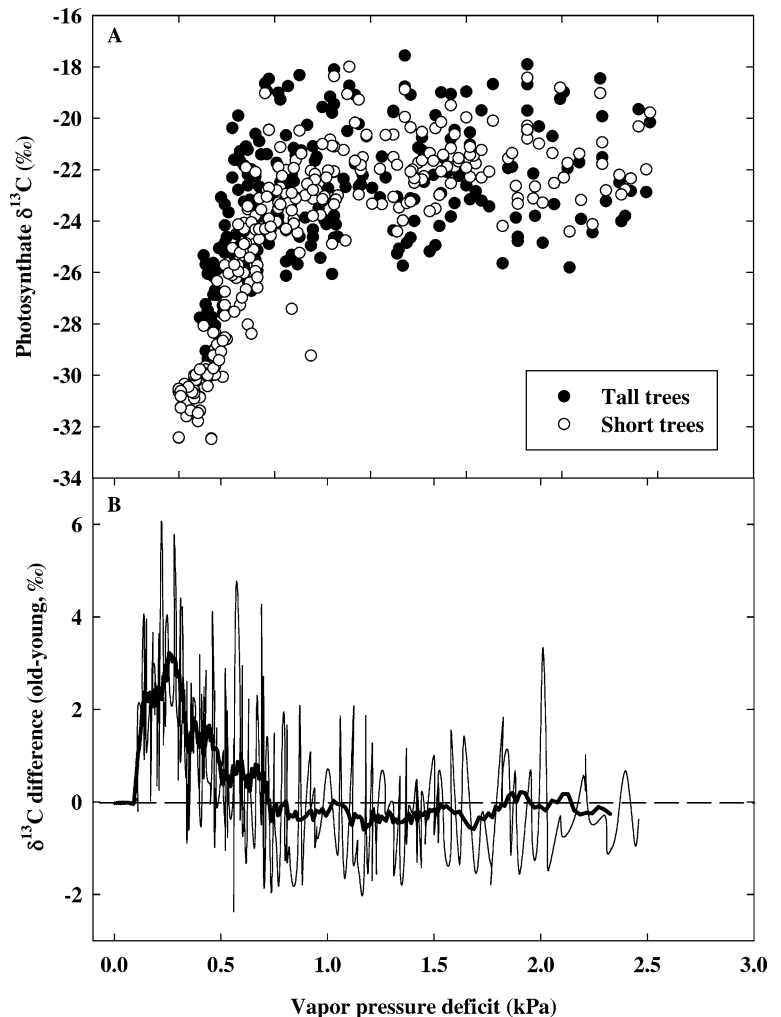

Fig. 9 Modeled predictions of foliar photosynthate $\delta^{13}C$ of the short and tall trees in relation to SWC. Weekly average values are plotted for clarity, and polynomial fits to the data are shown to highlight the trends. At SWC values above 0.22 g H_2O g soil^{-1}, the tall trees had significantly higher $\delta^{13}C$ than the short trees ($P = 0.01$), but there was no significant difference below 0.22 ($P = 0.31$). Note that the model predicts the short stand experiences lower SWC in the late summer (values below 0.20). This analysis supports the hypothesis encapsulated by Eq. 3 and Fig. 1b

due to water stress (Martin and Ruiz-Torres 1992; Escalona et al. 1999; Ellsworth 2000) which would act to increase Δ (decrease $\delta^{13}C$, Eqs. 1, 2). However, we predicted a decrease in Δ with increasing drought (Fig. 6a), opposite of that expected if declines in photosynthetic capacity were significant (see Eq. 1). This suggests that constraints on g_s greatly outweighed changes in photosynthetic capacity as has been seen for Douglas-fir in the Pacific Northwest (Panek and Waring 1997). Furthermore, no seasonal variation has been detected in photosynthetic capacity between the short and tall trees used in this study (McDowell et al. 2002a, Julia Kerrigan unpublished data).

Mesophyll resistance to CO_2 diffusion from the stomatal pore to the chloroplasts reduces the effective CO_2 concentration at the sites of carboxylation (Evans et al. 1986; Lloyd et al. 1992; Evans and von Caemmerer 1996; Gillon and Yakir 2000). This reduction in the functional c_i will decrease Δ below that expected by A and g alone (Eq. 2). However, while changes in g_i affected the absolute values of our modeled estimates of Δ, they had little effect on the seasonal patterns in the $\delta^{13}C$ difference between ages (Fig. 7). This suggests that our conclusions regarding environmental sensitivity of Δ are robust to

variation in g_i. The consistency between the simple hydraulic model (Fig. 1), *SPA* (Fig. 6), and the direct measurements of gas exchange (Figs. 2, 3, 4) allow us to conclude that, regardless of potential model parameterization errors, greater environmental sensitivity of Δ in short versus tall trees is a likely phenomenon in Douglas-fir.

Implications of size-dependent environmental sensitivity of gas exchange

The observation that stomatal sensitivity to D is proportional to maximum g_s (Kaufmann 1982, McNaughton and Jarvis 1991, Yong et al. 1997) suggests that plants with high maximum g_s may be more sensitive to climate changes that increase D. Consistent with this logic, our observations and those of Oren et al. (1999) suggest that trees with relatively low k_1 (e.g., tall trees) may be less sensitive to climate changes than those with high k_1 (e.g., short trees). Although numerous other factors differ with tree size that can interact with climate change, in its simplest form this theory suggests that incremental increases in D across a landscape will have a greater impact on carbon uptake of short-statured forests than of tall-statured forests. Including size-dependent environmental sensitivity of photosynthesis in process models may have a significant impact on predictions of future carbon uptake at regional scales.

Reduced maximum g_s and A found in the taller trees may result in reduced stem growth compared to shorter-statured forests (Gower et al. 1996; Ryan et al. 1997) and potentially limit net ecosystem CO_2 exchange. The majority of carbon assimilation by forests of the Pacific Northwest is in early summer when solar radiation is high yet soil water depletion is still minimal. Peak values of net ecosystem uptake based on eddy correlation measurements at the old-growth forest we studied occur during the wet, spring months (April–June, Paw et al. 2004). Since size-related differences in k_1 and subsequently g_s and A are primarily manifest during periods of mild environmental conditions, this implies that k_1 limitations to productivity are manifest when carbon assimilation potential is at its greatest (spring). Taking this conclusion a step further, we hypothesize that hydraulic limitations to gas exchange will be more manifest during periods of wetter weather compared to dry periods (e.g., La Niña versus El Niño events in the Pacific Northwest). Future research on size-dependent gas exchange should emphasize conducting measurements during periods when gross photosynthesis is highest in order to adequately capture the full magnitude of the effects of tree size on water use, carbon uptake, and isotopic discrimination.

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