

STABLE CARBON ISOTOPES AS INDICATORS OF LIMITATIONS TO FOREST GROWTH IMPOSED BY CLIMATE STRESS

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Abstract. Stable carbon isotopes have the potential to be sensitive indicators of carbon uptake limitations in forest trees. We assessed the ability of climate factors to explain the variation in $\delta^{13}\text{C}$ in foliage and annual rings of Douglas-fir trees at six sites along a steep climatic gradient in Oregon. To examine the physiological basis of the relationship between climate and $\delta^{13}\text{C}$, we parameterized a process-level model (FOREST-BGC) and used the model to separate the daily limitations on stomatal conductance due to climate factors. We found that, of the climatic constraints that could affect stomatal conductance (freezing temperature, humidity deficit, and soil drought), only the cumulative growing season constraints on stomata imposed by humidity deficit explained the variability in the carbon isotope composition of cellulose in foliage. Over an 8-yr period at any one site, modelled stomatal limitation due to humidity deficit was significantly correlated with foliar $\delta^{13}\text{C}$. Over the same period, $\delta^{13}\text{C}$ in annual growth rings was less well correlated with modelled stomatal constraints. This relation between $\delta^{13}\text{C}$ and absolute humidity demonstrated the sensitivity of carbon uptake in Douglas-fir, a species with a broad range across the intermountain West, to moisture at the leaf-atmosphere interface. Furthermore, the parameters derived from the relationship offer a baseline from which to assess limitations imposed on Douglas-fir carbon uptake from sources other than climate.

Key words: forest climate stress; forest modelling; *Pseudotsuga menziesii*; stable carbon isotopes; stomatal conductance; tree rings.

INTRODUCTION

Forests are an important part of the stability of the global ecosystem. They are major contributors to water and nutrient cycling, and they interact with the atmosphere and lithosphere, regulating climatic extremes and filtering atmospheric pollutants. They modify the form and appearance of landscapes and they also provide a number of products on which humans depend. Changes in climate, however, and the increase of anthropogenic pollutants threaten the condition of forested ecosystems. Monitoring the status of Earth's forests should be a priority in any effort to monitor the influence of changes on global ecosystems.

The sensitivity of a forest species to its changing environment may involve stress responses that limit the ability of the species to take up carbon. The effect of environmental constraints on carbon uptake in trees is often subtle, and the symptoms only become visible after a long period of cumulative stress. In this study, we identified the climatic limitations to which one forest species is sensitive, using stable carbon isotope abundance in tree foliage and growth rings as sensitive stress indicators. We examined Douglas-fir (*Pseudo-*

tsuga menziesii) across a broad climatic gradient to determine which of the many climate stresses was most important in limiting carbon uptake. We expect that the relationship we found will hold over the entire range of the species, thus serving as a baseline from which other stresses that affect the carbon uptake mechanism, e.g., ozone, SO_2 , and temporal climatic fluctuations, can be assessed.

Foliar $\delta^{13}\text{C}$ was analyzed, because stomata serve as the most direct link between the tree and its climatic environment, and are the site of carbon isotope uptake. We also analyzed isotopes in tree rings, which allowed for retroactive sampling as far back as trees could be aged. Although annual increment in tree growth has been used successfully to track significant long-term changes in forest response to temperature and precipitation when trees are located near their physiological limits (Fritts 1976), correlations between indices of tree ring width and climate are poor in mild climates (Meko et al. 1993). We used carbon isotopes because they were potentially a more sensitive indicator of limitations to carbon uptake in such environments, and because we sought a method that was generalizable to most environments.

Process-level forest growth models have been successful at explaining and predicting large-scale differences in observed tree growth, using daily and seasonal variation in solar radiation, atmospheric humidity deficits, temperature, and physiologically defined soil drought. One such model, FOREST-BioGeoChemistry

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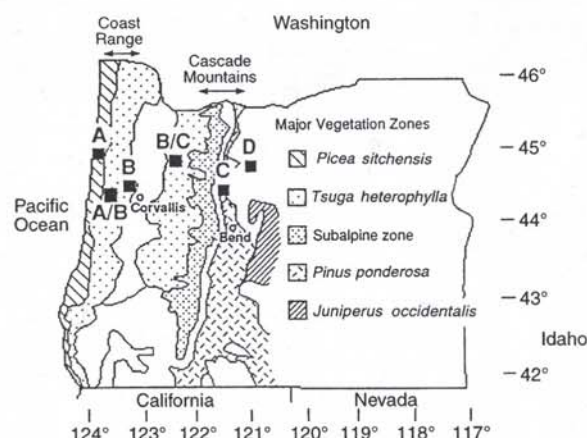


FIG. 1. Site locations across a steep climatic gradient in Oregon (from Panek and Waring 1995: Fig. 1).

(FOREST-BGC), was developed and tested using a large climatic gradient in the Pacific Northwest (Running and Coughlan 1988, Running 1994), a region relatively unaffected by air pollution away from the few population centers. This gradient and this model provided the opportunity to assess the ability of $\delta^{13}\text{C}$ to serve as an indicator of forest response to climate stress across a range of climates. The gradient provided the range of climate stresses to compare with observed variation in tree $\delta^{13}\text{C}$, and the model was used to understand the mechanisms through which climate imposed limitations to physiological processes.

The abundance of $\delta^{13}\text{C}$ in tree tissue changes in response to stresses that cause leaf stomata to open and close. During carbon fixation, carbon is twice fractionated, once as it diffuses into the leaf via the stomata, and once by the carbon-fixing enzyme, rubisco (ribulose biphosphate carboxylase/oxygenase). In both cases, the heavier isotope is discriminated against in favor of the lighter. Stomatal conductance and the rate of carbon assimilation control the internal CO_2 concentration and, thus, the uptake of ^{13}C . When internal CO_2 concentrations drop, as when stomata are constricted due to stress, rubisco discrimination declines and the ratio of $^{13}\text{C}/^{12}\text{C}$ in tree tissue rises. Thus, $\delta^{13}\text{C}$

provides an indication of how stress limits stomatal conductance and affects carbon fixation during cellulose formation (Farquhar et al. 1982, O'Leary 1993):

$$\delta^{13}\text{C}_{\text{leaf}} = \delta^{13}\text{C}_{\text{air}} - a - (b - a) \frac{c_i}{c_a}, \quad (1)$$

where c_i and c_a are the leaf internal and atmospheric CO_2 concentrations respectively, a is the isotopic discrimination from diffusion (4.4‰), and b is the discrimination from carboxylation (30‰).

Climate factors controlling water availability and atmospheric humidity are known to affect tree $\delta^{13}\text{C}$ abundance (Tans and Mook 1980, Freyer and Belacy 1983). More recently, however, the underlying physiological response of trees to climate has been investigated. Transpiration, estimated from a site water balance model, explained up to 68% of tree ring $\delta^{13}\text{C}$ variability in Douglas-fir (Livingston and Spittlehouse 1993). Strong correlations between stable carbon isotope abundance in foliage and branch hydraulic properties demonstrate that limitations to water movement through trees influence isotopic uptake (Waring and Silvester 1994, Panek and Waring 1995, Panek 1996).

We therefore investigated the ability of $\delta^{13}\text{C}$ to predict climatic limitations to carbon uptake, first by modelling the climate-related stomatal constraints with FOREST-BGC, and by measuring hydraulic constraints to stomata. Then, we established the relation between these constraints and $\delta^{13}\text{C}$ in tree tissue. Finally, we tested the power of this relation to predict $\delta^{13}\text{C}$ on an independent data set.

MATERIALS AND METHODS

Site descriptions

Six sites were chosen from a well-studied transect (Gholz 1982, Peterson and Waring 1994) that cuts across a steep climatic gradient in Oregon (Fig. 1, Table 1). Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) was common to all sites. Four sites were the same as those previously established. Two sites, A/B and D, were added to the transect to better represent zones of climate transition (Panek 1996). Site B/C is called

TABLE 1. Climate characteristics at the study sites in Oregon.

Site	Location	PPT† (cm)	Climate features
A	Cascade Head	243	Summer rain common, frequent fog, freezes rare
A/B	Marys Peak	164	Summers hot and dry, occasional midwinter freezes
B	Willamette Valley	106	Summers hot and dry, occasional midwinter freezes
B/C	Scio	136	Summers hot and dry, occasional midwinter freezes
C	Jefferson Wilderness	37	Rain shadow of Cascade Mountains, summers hot and dry, winters frozen
D	Grizzly Mountain	30	Rain shadow of Cascade Mountains, summers hot and dry, winters frozen

† PPT, annual average precipitation. Climate data are summarized from 1970–1994 records courtesy of the Oregon Climate Service, Corvallis, Oregon.

TABLE 2. Values used as initial conditions to parameterize FOREST-BGC for each site across the transect. Transpiration and T/PT are results from model runs, representing growing-season cumulative numbers averaged over 20 years (mean \pm 1 SE).

Site	Stem carbon (10 ³ kg/ha)	Leaf carbon (10 ³ kg/ha)	LAI† (m ² /m ²)	T/PT‡	Cumulative transpiration (mm)
A	317	3.4	12.2	0.80 \pm 0.01	187 \pm 12
A/B	207	4.3	10.7	0.79 \pm 0.02	187 \pm 5
B	207	4.3	10.7	0.79 \pm 0.02	203 \pm 7
B/C	165	3.4	8.6	0.78 \pm 0.01	294 \pm 12
C	6	1.0	1.6	0.52 \pm 0.02	63 \pm 1
D	4	1.5	1.2	0.45 \pm 0.02	96 \pm 1

† LAI, leaf area index.

‡ Transpiration as a proportion of potential transpiration, measured as water per growing season. The growing season was defined as beginning when modelled carbon gains exceeded those lost through respiration, and ending at budset.

level, attenuated by a Beer's extinction coefficient of 0.5 through the given leaf area index of the canopy. Conductance is then constrained sequentially by functions of canopy leaf water potential and absolute humidity deficit. The canopy leaf water potential is derived from a function of soil water content and capacity. Finally, subfreezing temperatures constrain canopy conductance for the 24 h following return to above-freezing conditions. Transpiration is determined using a Penman-Monteith equation modified for conifer needle geometry. FOREST-BGC requires species and site characteristics and daily climate data as input (temperature, precipitation, relative humidity, solar radiation). Site parameters have been determined from previous work at four of the six sites in this study (Running 1994). Parameters for the two new sites were estimated from the closest parameterized site. Relevant parameters are listed in Table 2.

For every site, stomatal constraint due to humidity deficit (vapor pressure deficit, VPD), soil drought, or freezing temperature, was quantified by computing the sum of reductions in conductance and comparing these to maximum stomatal conductance at the same irradiance (Panek and Waring 1995). An index of overall limitation to gas exchange was estimated with the ratio of modelled transpiration to the potential transpiration without stomatal constraint (T/PT). Stomatal constraint and T/PT were calculated daily, and summed annually, for 20 years of climate data. Temperature and precipitation data were obtained from the Oregon State Climate Center (1975–1994; G. Taylor, Oregon Climate Center, Oregon State University, Corvallis, Oregon). Absolute humidity and solar radiation were modelled from these data using the climate model MT-CLIM (Glassy and Running 1994). We validated the output from this model on a 1990 data set from sites across the transect. Measured and modelled values were not significantly different from each other ($P < 0.05$).

In some species of tree, starch reserves from the

previous year contribute to the construction of the current year's leaves, complicating the relationship between foliar $\delta^{13}\text{C}$ and climate. In Douglas-fir, the current year's growth is derived exclusively from the current year's photosynthate. Starch reserves from the previous year are not utilized for foliar growth (Webb and Kilpatrick 1993). The modelled growing season was defined to begin when the current year's starch reserves began to accumulate, i.e., when modelled photosynthesis exceeded modelled respiration. The end of the growing season was defined as 20 August, the date by which budset had been observed at all sites.

Statistical analysis

The relationship between $\delta^{13}\text{C}$ and climatic constraints on stomata, within year and across sites, was determined using multiple linear regression with stepwise selection to find the best fit model from all possible models, using the variables VPD, soil drought, temperature, or, separately, the ratio of actual to potential transpiration, T/PT. Humidity deficit, drought, and temperature specify the nature of the constraint more precisely than does T/PT. The results have been reported previously for the between-site comparisons of foliage age classes 1990–1994 (Panek 1996), but not for tree rings.

Within any one site, $\delta^{13}\text{C}$ was highly autocorrelated between years, in both foliage and tree rings. Therefore, to model the within-site relationship between $\delta^{13}\text{C}$ and climatic constraints to stomata, it was necessary to account for the correlation between years and to incorporate the correlation structure into the analysis. We used a mixed linear model, which is a generalization of the standard linear model that allows for the analysis of data with several sources of variation, in this case, time and climatic constraints (SAS Institute 1992). If the autocorrelation between years is not accounted for, the correlation coefficient between $\delta^{13}\text{C}$ and climate factors can be spuriously high. Data were averaged by site, within year, to develop a regression model relating foliar and tree ring $\delta^{13}\text{C}$ to climatic constraints on stomatal conductance through time. Model fit was determined with the Akaike's Information Criterion (AIC). The model with largest AIC value (in this case, the least negative) given the same number of model parameters is the better fitting model (SAS Institute 1992). Variables were tested for significance at the 0.05 level. The predictive power of the resulting regression model was then tested on an independent data set created by jackknifing, that is, by moving observations from the full data set one at a time and using the remaining observations to model the deleted observation. The predicted value is then independent of the measured values.

RESULTS

Variation in $\delta^{13}\text{C}$

Foliar carbon isotope values ranged from -21‰ to -27‰ across the transect, and were significantly less

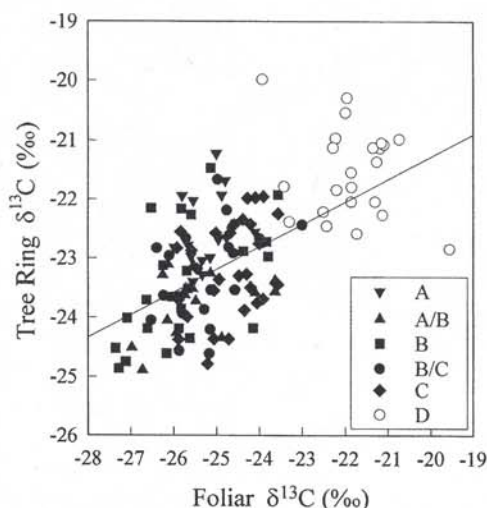


FIG. 4. Foliar $\delta^{13}\text{C}$ and tree ring $\delta^{13}\text{C}$, compared by year within tree, were linearly related, although the relation was not strong ($R^2 = 0.42$, $P < 0.0001$). Tree ring $\delta^{13}\text{C}$ was, on average, 1.7‰ heavier than foliage $\delta^{13}\text{C}$.

specific conductivity, $1/\kappa_s$ ($P < 0.0001$, $R^2 = 0.73$). Tree ring $\delta^{13}\text{C}$ variability across sites within years 1987–1994 was best explained by a multiple regression with $1/\kappa_s$ ($P < 0.0001$) and VPD ($P = 0.03$), $R^2 = 0.66$.

Within each site, assessing the variation over time, the mixed model that best explained the variation in foliar $\delta^{13}\text{C}$ included only the variable VPD (Fig. 5, $P = 0.0004$, AIC = -41.1). A model including only T/PT was nearly as good at explaining foliar $\delta^{13}\text{C}$ variation within site ($P = 0.0008$, AIC = -37.9), but T/PT is a function of VPD. Hence, the VPD model was more specific in explaining the source of variation. Neither soil drought nor freezing temperature was a significant variable. The model which best described within-site variability in tree ring $\delta^{13}\text{C}$ was T/PT alone ($P = 0.0012$). Model parameters are presented in Table 3.

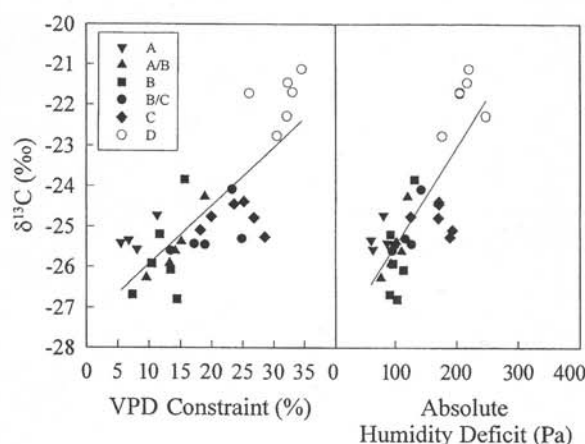


FIG. 5. The relation between foliar $\delta^{13}\text{C}$ abundance and modelled stomatal constraint due to vapor pressure deficit (VPD), and between foliar $\delta^{13}\text{C}$ and absolute humidity deficit. These relations form the basis of the models to predict foliar $\delta^{13}\text{C}$.

We know of no other study that has taken into account autocorrelation of the isotopic signatures between foliage age classes or growth rings by using a mixed model. The procedure is analogous to detrending tree growth ring widths before comparing them to climate variables. Although the Akaike's Information Criterion is not as familiar as the R^2 , we believe that this statistical analysis avoids spuriously high correlation results. If, like many reported studies, we use a multiple linear regression to compare $\delta^{13}\text{C}$ to stomatal constraints after accounting for variation due to site, VPD explains 87% ($P < 0.0001$) of foliar $\delta^{13}\text{C}$ variability, and T/PT explains 72% ($P < 0.0001$) of tree ring $\delta^{13}\text{C}$ variability, mostly due to autocorrelation between years.

The results of the model analysis indicate that VPD is the primary constraint on stomata in Douglas-fir. It follows that, at each site, the recorded climate factor, absolute humidity deficit (ABSHD), can then be used

TABLE 3. Parameters for the regression model relating $\delta^{13}\text{C}$ to climate constraints on stomata in Douglas-fir within each site through time.

Parameter	Estimate	1 SE	P	AIC†
A) Foliar $\delta^{13}\text{C}$				-41.1
Modelled climate constraint				
VPD (proportion of maximum conductance)	0.11	0.02	0.0004	
Intercept	-26.77	0.62	0.0001	
Measured climate variable				-38.6
ABSHD (Pa)	194	47.9	0.0004	
Intercept	-27.2	0.71	0.0001	
B) Tree ring $\delta^{13}\text{C}$				-39.7
T/PT	-3.95	1.13	0.001	
Intercept	-20.16	0.82	0.0001	

Note: Column 1 abbreviations are VPD, vapor pressure deficit; ABSHD, absolute humidity deficit; T/PT, the ratio of modelled transpiration to the potential transpiration without stomatal constraint.

† Akaike's Information Criterion. See *Statistical analyses*.

can occur. The variation in foliar $\delta^{13}\text{C}$ values was equally well explained by the transpiration index, which is an integrated measure of all constraints to stomata. This implied that the major factor limiting stomatal conductance and, thus, carbon uptake and transpiration, was the stomatal constraint caused by low atmospheric humidity.

The $\delta^{13}\text{C}$ values predicted by modelled stomatal constraint and by measured absolute humidity deficit at each site corresponded quite well to the within-site $\delta^{13}\text{C}$ values observed over years. The exception was site D. Here, the $\delta^{13}\text{C}$ values observed were higher than those predicted. The results of the hydraulic conductivity analysis showed that the interior Douglas-fir variety at this site had significantly lower specific and leaf-specific conductivity. The limitations that this imposed on water movement through the branches constrained the stomata in a manner not directly related to climate (Pannek 1996), and could explain the poor fit of the model at this site. To predict $\delta^{13}\text{C}$ across a range of geographic locales, then, requires taking into account not only direct climate factors but also the physiological changes that those climate factors produce. In this case, the lower hydraulic conductivity of the interior variety of Douglas-fir is an adaptation to the drier climate of the intermountain West. This suggests that a separate relation between climate constraints to stomata and $\delta^{13}\text{C}$ exists for each species of tree, and possibly for each variety as well, based on its hydraulic conductivity. Thus, using foliar $\delta^{13}\text{C}$ as a stress indicator is more useful within site over time than over a broad climatic region, unless one also measures branch hydraulic conductivity. Although site nitrogen status has been shown to affect maximum stomatal conductance across a broad range of species (Schulze et al. 1994), the nitrogen status of the sites probably did not contribute to isotopic variation, since there is little variation in the foliar nitrogen content between sites (Matson et al. 1994), and the same species of tree was used across all sites.

A significant amount of tree ring $\delta^{13}\text{C}$ variation was explained by the transpiration index, although the model fit was not as good as that for foliar $\delta^{13}\text{C}$. None of the specific climatic constraints, freezing temperature, soil drought, or VPD, was significant in the model. Our results corroborated the findings of Livingston and Spittlehouse (1993), who also reported a correlation between tree ring $\delta^{13}\text{C}$ and transpiration in Douglas-fir. Because none of the specific climate factors explained the variation in $\delta^{13}\text{C}$, our results further suggested that other sources of variation beyond climate contribute to the variation in tree ring $\delta^{13}\text{C}$. Tree ring $\delta^{13}\text{C}$ is not as tightly coupled to environmental factors that constrain stomata as is foliar $\delta^{13}\text{C}$. $\delta^{13}\text{C}$ is fractionated during translocation and wood-cellulose formation, and is thus subject to processes unrelated to climate (Deines 1980, O'Leary 1981). Furthermore, wood production continues after foliar elongation has ceased, and thus inte-

grates a different climate signal (Emmingham 1977). This difference in growing season was not accounted for during modelling, in part because springwood represents the majority of tree ring growth. Any resultant bias, however, would appear as an underestimation of tree ring $\delta^{13}\text{C}$ abundance. Sampling only from one aspect probably did not contribute to the weak relationship between climate factors and tree ring $\delta^{13}\text{C}$, or between foliar and tree ring $\delta^{13}\text{C}$. Although there is significant circumference variability in tree ring $\delta^{13}\text{C}$, trends in $\delta^{13}\text{C}$ from ring to ring are consistent, regardless of aspect (Tans and Mook 1980, Leavitt and Long 1986). In this study, only the south sides of trees were sampled, with the objective of maximizing the chance of discerning yearly variation in $\delta^{13}\text{C}$ attributable to environmental factors. For foliage on branches, this approach was appropriate, because each branch is essentially carbon autonomous (Sprugel et al. 1991). Wood production in the tree stem, however, integrates the photosynthate from a large, but unknown, portion of the crown, favoring regions with higher stomatal conductance. Thus, the wood in the south side of the stem may not represent processes on the south side of the crown (Vité and Rudinsky 1959).

Relation between tree ring $\delta^{13}\text{C}$ and tree ring width

Ring width indices were not correlated with tree ring $\delta^{13}\text{C}$. Both tree ring width and $\delta^{13}\text{C}$ represent indices of carbon-fixing processes, but in different ways. Ring width represents the carbon remaining for increment growth after fixed carbon is used for fine roots, shoots, injury repair, and reproduction (Kramer and Kozlowski 1979, Waring and Schlesinger 1985). $\delta^{13}\text{C}$, on the other hand, is a permanent chemical signature of limitations on carbon fixation. Translocated carbon is further fractionated before it is incorporated into tree ring tissue. As earlier dendrochronology studies have demonstrated, growth increment is a poor measure of environmental variation in Pacific Northwest forests (Meko et al. 1993).

Predictive capabilities

The results of this study have demonstrated that the uptake of carbon in Douglas-fir is sensitive to changes in the humidity of the surrounding environment on a seasonal time scale. Within a site, low humidity leads to stomatal constraint and, thus, to limitation in carbon assimilation. It follows that among the responses of forests to local disturbances, for example, large gaps in the canopy caused by clear-cutting, fire, or insect damage, lowered humidity contributes to photosynthetic limitations in the remaining trees. Many factors change after disturbance, including soil nitrogen availability, affecting forest growth response. The response of trees to changes in humidity should be considered as one among many responses.

On a larger scale, a doubling of global atmospheric CO_2 concentrations is projected to induce climate

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