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Vertical canopy gradients in $\delta^{13}\text{C}$ correspond with leaf nitrogen content in a mixed-species conifer forest

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Abstract Stable carbon isotope composition varies markedly between sun and shade leaves, with sun leaves being invariably more enriched (i.e., they contain more ^{13}C). Several hypotheses have emerged to explain this pattern, but controversy remains as to which mechanism is most general. We measured vertical gradients in stable carbon isotope composition ($\delta^{13}\text{C}$) in more than 200 trees of nine conifer species growing in mixed-species forests in the Northern Rocky Mountains, USA. For all species except western larch, $\delta^{13}\text{C}$ decreased from top to bottom of the canopy. We found that $\delta^{13}\text{C}$ was strongly correlated with nitrogen per unit leaf area (N_{area}), which is a measure of photosynthetic capacity. Usually weaker correlations were found between $\delta^{13}\text{C}$ and leaf mass per area, nitrogen per unit leaf mass, height from the ground, or depth in the canopy, and these correlations were more variable between trees than for N_{area} . Gradients of $\delta^{13}\text{C}$ (per meter canopy depth) were steeper in small trees than in tall trees, indicating that a recent explanation of $\delta^{13}\text{C}$ gradients in terms of drought stress of upper canopy leaves is unlikely to apply in our study area. The strong relationship between N_{area} and $\delta^{13}\text{C}$ here reported is consistent with the general finding that leaves or species with higher photosynthetic capacity tend to maintain lower CO_2 concentrations inside leaves. We conclude that photosynthetic capacity is a strong determinant of $\delta^{13}\text{C}$ in vertical canopy profiles, and must be accounted for when interpreting $\delta^{13}\text{C}$ values in conifer forests.

Keywords Carbon stable isotopes · Acclimation · Photosynthetic capacity · Species variation · Mixed-species conifer forest

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Introduction

The ratio of ^{13}C to ^{12}C can be used to infer past gas-exchange characteristics of plant canopies. Although photosynthesis strongly discriminates against ^{13}C , this discrimination is expressed to varying degrees among leaves, resulting in variation in $^{13}\text{C}/^{12}\text{C}$ (expressed as $\delta^{13}\text{C}$) across species (Marshall and Zhang 1994), across years with varying drought intensity (Guehl et al. 1991), within growing seasons (Fessenden and Ehleringer 2003), and with short-term fluctuations in air humidity (Bowling et al. 2002). Pronounced vertical gradients in $\delta^{13}\text{C}$ have also been described in many forest canopies (Broadmeadow and Griffiths 1993).

The mechanism controlling the variation in tissue $\delta^{13}\text{C}$ is the balance between outward and inward diffusion of CO_2 through the stomata, which is in turn controlled by the ratio of intercellular CO_2 concentration (c_i) to ambient CO_2 (c_a) (cf. Farquhar et al. 1982). This ratio is controlled by the supply of CO_2 through the stomata and demand for it in the photosynthetic tissues of the leaf mesophyll. Many studies have correlated variation in tissue $\delta^{13}\text{C}$ with stomatal conductance, where stomatal conductance controls the diffusive supply of CO_2 into the leaves.

Within plant canopies, there often exists a large variation in $\delta^{13}\text{C}$. Generally, sun leaves contain more ^{13}C than shade leaves (less negative $\delta^{13}\text{C}$), indicating that sun leaves operate at lower $[\text{CO}_2]$ inside chloroplasts than shade leaves (review by Broadmeadow and Griffiths 1993 and Le Roux et al. 2001). The exact mechanism behind this variation remains controversial (Le Roux et al. 2001; Niinemets et al. 2004a), because it cannot be easily explained by stomatal closure alone.

Schleser and Jayasekara (1985) demonstrated 5‰ within-canopy variation in $\delta^{13}\text{C}$, and attributed the gradient to assimilation of soil-respired CO_2 , which is generally depleted in ^{13}C . This mechanism is unlikely to have any serious effect on $\delta^{13}\text{C}$ however, since soil-respired CO_2 does not have a noticeable effect on ambient CO_2 above a height of 1 m above the forest soil (Buchmann et al. 2002). Since it was

realized that respiratory CO_2 has likely little influence on plant $\delta^{13}\text{C}$, most studies have attributed the $\delta^{13}\text{C}$ gradient to the direct effect of environmental gradients within the canopy. Francey et al. (1985), Zimmermann and Ehleringer (1990), and Le Roux et al. (2001) suggested that the vertical gradient in $\delta^{13}\text{C}$ is the result of the direct effect of irradiance, which varies strongly within the canopy.

In a detailed study, Niinemets et al. (2004a) attributed the c_i/c_a gradient to gradients of drought stress within the canopy. Diurnal changes in stomatal conductance were stronger at the top of the canopy, causing drawdown of c_i relative to shade leaves. Sun leaves may be more water stressed for three reasons. First, vapor pressure deficit is potentially higher for sun leaves, a major constraint on stomatal opening. For forest canopies (and especially conifer canopies), such gradients are however minimal (Jarvis et al. 1976). Secondly, higher leaf temperature would increase the leaf-to-air vapor pressure difference (Martin et al. 1999). Leaf temperature gradients are not necessarily always in the same direction (Martin et al. 1999), because both radiation load (higher at the top of the canopy) and boundary layer conductance (lower at the bottom of the canopy) control leaf temperature (Jones 1992). Finally, if the canopy is sufficiently tall, limitation to water transport may cause gradients in leaf water potential (Ryan and Yoder 1997; Hubbard et al. 1999). This so-called hydraulic limitation may in turn cause gradients in stomatal closure within the canopy. If this mechanism is responsible for gradients in c_i , taller trees should show larger difference in $\delta^{13}\text{C}$ between sun and shade leaves, but the slope (‰cm^{-1}) should be similar.

Photosynthetic capacity (A_{max}) varies strongly within forest canopies (e.g., Bond et al. 1999). Leaf nitrogen is a reliable indicator of A_{max} (see Duursma et al. 2005 for a review of coniferous species), especially when gradients within the canopy are considered (e.g., Niinemets et al. 1998). Leaf mass per area is also an indicator of A_{max} when mass-based nitrogen concentration (N_{mass}) is relatively constant, as is the case within conifer canopies (Niinemets 1997a; Bond et al. 1999; Palmroth and Hari 2001). Many studies have demonstrated strong correlations between N_{area} and $\delta^{13}\text{C}$, or LMA and $\delta^{13}\text{C}$. This is true not only when variation in LMA or N_{area} comes from within canopy variation (Hanba et al. 1997; Niinemets et al. 1999; Roberts et al. 1999; Turney et al. 2002; Koch et al. 2004), but also altitude transects (Vitousek et al. 1990; Sparks and Ehleringer 1997; Cordell et al. 1999; Hultine and Marshall 2000; Li et al. 2004), site-to-site variation in water availability (Lamont et al. 2002; Samuelson et al. 2003; MacFarlane et al. 2004), and in some cases even species or provenances (Wright et al. 1993; Zhang and Marshall 1995; Monclus et al. 2005 only with N_{area} not LMA). This indicates that photosynthetic capacity is potentially an important regulator of $\delta^{13}\text{C}$ variation among leaves, and may explain gradients of $\delta^{13}\text{C}$ within canopies (Hanba et al. 1997).

First, we hypothesized that the vertical $\delta^{13}\text{C}$ gradient within conifer canopies is more strongly related to N_{area} (an estimate of A_{max}) than height or canopy depth. Second, we hypothesized that if increased hydraulic limitation in

upper canopy leaves causes increased stomatal limitation in sun leaves versus shade leaves, this effect would be more pronounced in tall trees than in short trees. Finally, because N_{area} varies between our study species (Duursma et al. 2005), it may be expected that difference between species in $\delta^{13}\text{C}$ is partly due to differences in N_{area} . We test these hypotheses with two datasets describing canopy gradients from more than 200 trees of nine conifer species in northern Idaho, USA.

Methods

Foliar data came from two separate sampling efforts in the Priest River Experimental Forest (PREF). The first study will be denoted by PREF94 because sampling occurred in 1994 (Monserud and Marshall 1999; Marshall and Monserud 2003). The second study will be denoted PREF01 because sampling occurred in 2001 (Duursma et al. 2005). Detailed descriptions of the sampling designs are presented later.

Study area

The Priest River Experimental Forest (PREF) is located in the panhandle of northern Idaho, USA ($48^\circ 21' \text{N}$, $116^\circ 45' - 116^\circ 50' \text{W}$). The PREF climate is transitional between a northern Pacific coastal and continental type (Finklin 1983). Twelve conifer species occur naturally in the PREF. Species composition in these forests depends to a large extent on aspect and altitude (Cooper et al. 1991). The nine sampled species were western red-cedar (*Thuja plicata* Donn ex D. Don), western hemlock (*Tsuga heterophylla* (Raf.) Sarg.), the interior variety of Douglas-fir (*Pseudotsuga menziesii* var. *glauca* (Mirb.) Franco), grand fir (*Abies grandis* (Dougl. ex D. Don) Lindl.), western white pine (*Pinus monticola* Dougl. ex D. Don), western larch (*Larix occidentalis* Nutt.), ponderosa pine (*Pinus ponderosa* Dougl. ex P. & C. Laws.), subalpine fir (*Abies lasiocarpa* (Hook.) Nutt.), and lodgepole pine (*Pinus contorta* Dougl. ex Loud.).

Sampling design

PREF01

The experimental forest was stratified into three altitude and three solar insolation classes. We then randomly allocated thirty-six $90 \text{ m} \times 90 \text{ m}$ plots within these strata. Detailed descriptions of the plot allocation process are provided by Duursma et al. (2003) and Pociwicz et al. (2004). We sampled 22 of the 36 randomly located plots in the summers of 2001 and 2002. Trees at five points within the plots (from previous sampling, Duursma et al. 2003), were sampled for foliage. At each sampling point, one sampling tree was selected for sampling with the use of standard climbing equipment. The closest dominant or co-dominant tree to

the sampling point was chosen, but excluding obviously diseased or excessively leaning trees. When possible, one or two nearby trees were sampled from the climbed tree with the use of a 4 m pruning pole. All species were found suitable for climbing, but in many cases western larch and lodgepole pine were avoided. Late in the second sampling season, foliage samples from these species were taken at four plots using a rifle. The goal was to sample six shoots from each tree, including the climbed tree as well as the adjacent trees. The crown was visually stratified into low, middle, and top canopy, and two shoots were sampled from each third. The actual heights of the samples were measured with a measure tape attached to the climber. Within each crown third, the two samples were taken from the same height, but from diametrically opposed main branches. In total, 170 trees of nine different species were sampled.

Leaf mass per area

For determination of leaf mass per area, last year's fully expanded foliage was used except for cedar and larch. Western red-cedar does not show distinct annual shoots (Harlow et al. 2005), so instead the last 10 cm of the shoot, including the apex and all green foliage sprouting from the main shoot, was processed. For the deciduous western larch, one short-shoot, which typically carried 20–30 needles, was used. The foliage was placed on a digital scanner. Projected area was estimated from the images with SigmaScan Pro (Aspire Software, Leesburg, VA, USA).

PREF94

Monserud and Marshall (1999) and Marshall and Monserud (2003) sampled three species (Douglas-fir, western white pine, ponderosa pine) in eight mixed-species stands in the PREF (see description of the PREF earlier). A total of 58 trees were harvested, and eight branches were sampled from each tree. The eight branches were sampled such that two from each quarter were a randomly chosen pair of antithetic variates; this process distributed the samples vertically throughout the crown. For each branch, a subset of 10–25 current-year needles near the end of the branch was collected. For these samples, leaf mass per area is available (Marshall and Monserud 2003); we analyzed the same samples for N_{mass} and $\delta^{13}\text{C}$.

Stable carbon isotope composition

Bulk foliar samples were processed at the University of Idaho Stable Isotope Laboratory. Foliar samples were oven-dried at 70°C for at least 72 h and ground to a fine powder in a ball mill. N_{mass} (% g N/100 g dry weight) was determined with a CN-2400 EA (Carlo Erba) coupled to a Finnigan-MAT delta +. N_{area} (g N m⁻²) was determined as the product of LMA and N_{mass} /100. We report the standard δ notation, $\delta^{13}\text{C} = 1000(R_{\text{sample}}/R_{\text{standard}} - 1)$ (‰), where

R_{sample} is the $^{13}\text{C}/^{12}\text{C}$ of the sample, and R_{standard} the ratio for a standard (Pee-Dee belemnite). We report $\delta^{13}\text{C}$ (not discrimination), because it represents the raw data with a minimum of assumptions.

Data analysis

We tested the explanatory power of several different predictor variables for the variation in $\delta^{13}\text{C}$ within canopies. For significance testing, and to assess the variation between trees, we used a mixed-effects model of the form:

$$\delta^{13}\text{C} = (\beta_0 + b_0) + (\beta_1 + b_1) \log(X) + \varepsilon, \quad (1)$$

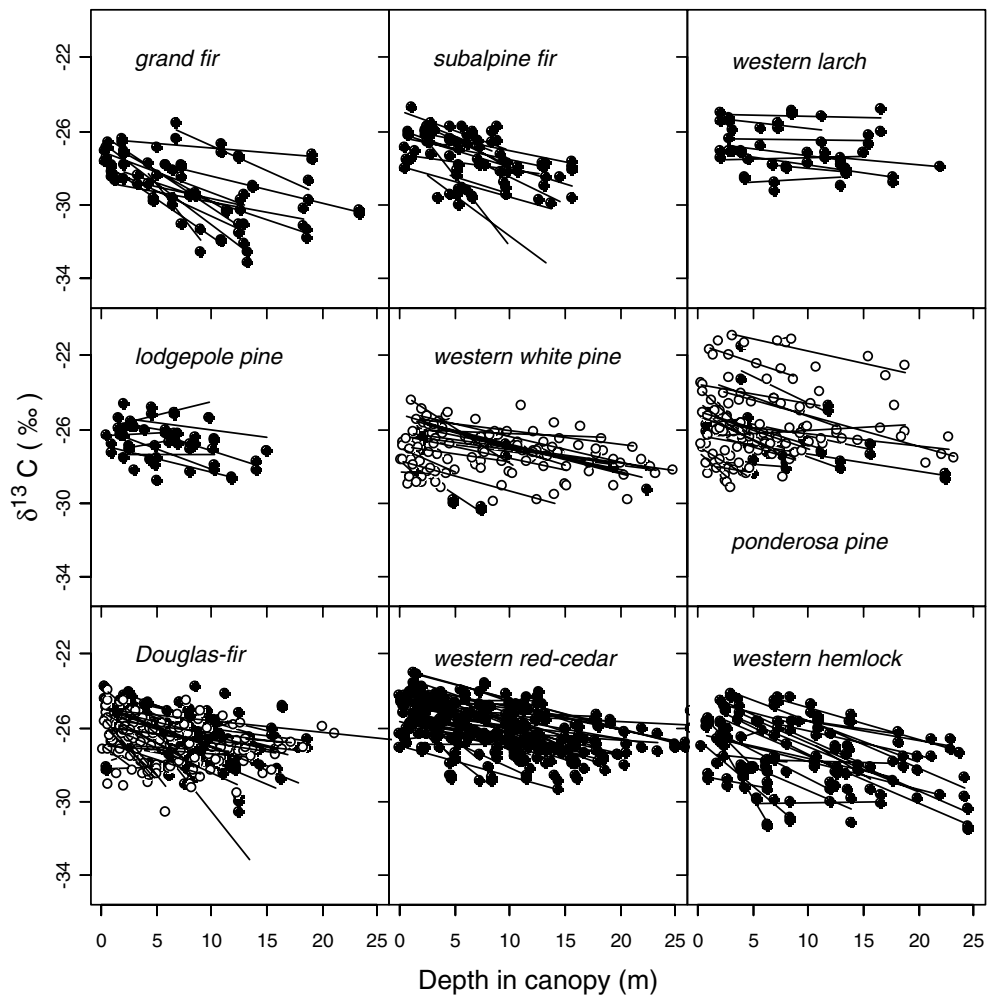
where β_0 and β_1 are the intercept and slope of the mean regression between $\delta^{13}\text{C}$ and X , b_0 , and b_1 the random effects that account for variation between trees and sites (with mean equal to zero, but with finite variances that can be correlated to each other), ε an error term (with components for site and tree levels). Log transformation of the independent variable was necessary in each case to meet model assumptions (Pinheiro and Bates 2000). The variance of the random effects ($\sigma_{b_0}^2$, $\sigma_{b_1}^2$) can be interpreted as the variability between trees in the relationship between $\delta^{13}\text{C}$ and some predictor variable. If this variance would be zero, the relationship between the two variables would be of the exact same form for each tree, an evidence that the relationship is more general. We report the coefficient of variation of the slope random effect (σ_{b_1} , β_1) as a measure of how general the relationship between $\delta^{13}\text{C}$ and some predictor variable is across trees. To estimate “goodness-of-fit”, we fitted a simple linear regression ($\delta^{13}\text{C} = \beta_0 + \beta_1 X$) for each tree separately, and estimated the average goodness-of-fit for all trees, species by species. For the independent variable (X), we used (1) N_{area} , (2) LMA, (3) N_{mass} , (4) depth in canopy, (5) height from the ground.

We used R^2 as the measure of goodness-of-fit; it was estimated as $1 - \sigma_{\text{error}}^2 / \sigma_Y^2$ where σ_Y^2 is variance of the dependent variable, and σ_{error}^2 the error variance (Kvålseth 1985). The average R^2 for a species was estimated as $1 - \overline{\sigma_{\text{error}}^2} / \overline{\sigma_Y^2}$, where the over-bars denote averages (this is possible because variances are additive).

To test the effect of species on the relationship between N_{area} and $\delta^{13}\text{C}$, we used the same mixed-effects model as in Eq. (1), but adding species as a fixed effect. First, we fit the full model with species effects on both intercept and slope, and second a simpler model with the species effect only on the intercept. These two models were tested against each other and against the model without the species effect on the intercept (Eq. (1)). These tests relied on Akaike's Information Criterion (AIC), root mean squared error (RMSE), and the usual significance testing of intercept and slopes.

Finally, we tested the effect of N_{area} on $\delta^{13}\text{C}$ after removing the variation in N_{area} levels between trees and species, as follows. For each tree, we divided N_{area} and $\delta^{13}\text{C}$ of each sample by N_{area} or $\delta^{13}\text{C}$ of the sample closest to the ground. We then tested whether this relative $\delta^{13}\text{C}$ was related to

Fig. 1 Gradients of $\delta^{13}\text{C}$ within the canopy for nine conifer species. Each line is a regression line of $\delta^{13}\text{C}$ against depth in canopy (meters from tree top) for each tree separately. Closed circles: PREF01 dataset, open circles: PREF94. Note the large variation in intercept ($\delta^{13}\text{C}$ at zero depth in canopy) among trees, but the consistent decline of $\delta^{13}\text{C}$ within the canopy. Note also the absence of these gradients in western larch



relative N_{area} in a general way, i.e., whether species was significant (in the same way as for $N_{\text{area}} - \delta^{13}\text{C}$, see earlier).

Results

Individual trees varied considerably in $\delta^{13}\text{C}$ at the top of the canopy (Fig. 1), but in almost every individual $\delta^{13}\text{C}$ decreased from the top of the crown toward the bottom. Western larch, the only deciduous species studied here, was the sole exception to this rule; it did not show the vertical gradient from top to bottom of the crown (Fig. 1).

For each species, $\delta^{13}\text{C}$ was significantly correlated to N_{area} (Fig. 2). The regression fitting these data was frequently curvilinear, approaching an asymptote at high N_{area} . For ponderosa pine, the relationship was rather weak overall (Fig. 2), but this was mainly the result of large variation among trees, which differed strongly in intercept values.

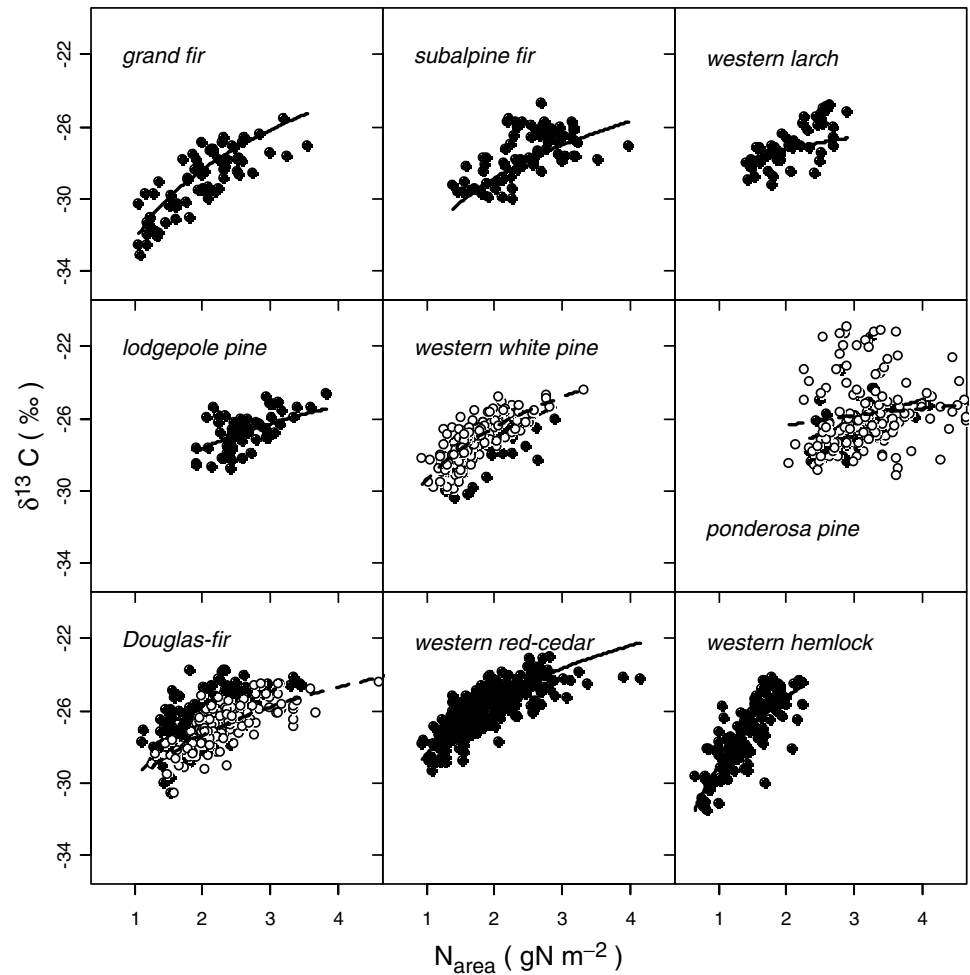
Several leaf characteristics were correlated with variation in $\delta^{13}\text{C}$ within individual tree crowns after tree-to-tree variation had been accounted for. N_{area} , LMA, depth in canopy, and height from ground all explained approximately equal amounts of the residual variation in $\delta^{13}\text{C}$ (R^2 in Table 1). This similarity occurred because these predictor variables

are correlated, e.g., N_{area} and LMA are nearly proportional because N_{mass} varies little within these canopies (Duursma et al. 2005). The correlations were especially strong for grand fir, in which 88% of variation in $\delta^{13}\text{C}$ was explained by LMA and 86% was explained by N_{area} . The correlations were also particularly strong in western red-cedar, in which 78% of the variation was explained by N_{area} . For the other species, this proportion was between 50 and 70%. N per unit leaf mass (N_{mass}) almost always explained less variation in $\delta^{13}\text{C}$ within crowns than N per unit leaf area (N_{area}). Western larch was again the exception to this rule.

The relationships between $\delta^{13}\text{C}$ and N_{area} or LMA were however much more consistent between trees than for the other variables (low variance of the random slope effect; Table 1). This implies that N_{area} or LMA, both more direct measures of photosynthetic capacity, are more directly related to variation in $\delta^{13}\text{C}$ than the other predictor variables.

For the PREF94 dataset, the gradients of $\delta^{13}\text{C}$ were significantly correlated to total tree height for ponderosa pine and Douglas-fir (Fig. 3a). The shorter trees showed a steeper decrease of $\delta^{13}\text{C}$ from top to the bottom of the crown than did the taller trees. Western white pine showed no such change in the vertical gradient. No such correlation was found in any species in the PREF01 dataset, which did

Fig. 2 Relationship between N_{area} and $\delta^{13}\text{C}$ for each species separately. *Black circles:* PREF01, *white circles:* PREF94. *Solid black line:* PREF01 (mixed-effects model), *dashed black line:* PREF94



not include small open-grown trees. The difference in $\delta^{13}\text{C}$ of leaves at the top of the canopy and those at the bottom was not related to total tree height in the PREF94 dataset for ponderosa pine or white pine (Fig. 3b), showing that small trees had the same amount of variation in $\delta^{13}\text{C}$ than large mature trees. For Douglas-fir, small trees showed a larger sun-shade difference than large trees, opposite to what was expected (Fig. 3b).

Species differed strongly in the intercept of the linear regression between $\delta^{13}\text{C}$ and $\log(N_{\text{area}})$, as can be seen from the large decrease in AIC and RMSE when including a species-specific intercept into Eq. (1) (Table 2). When a species-specific slope was added, however, AIC and RMSE decreased only very marginally (RMSE decreased less than 0.5%), showing that one general slope fits the data as well as separate slopes per species. Figure 4 shows the model fit with one general shape, but different intercept values. For a given N_{area} , $\delta^{13}\text{C}$ was quite different among species, but for unit increase in N_{area} each species responds similarly in $\delta^{13}\text{C}$ increase, although this increase is lower at high N_{area} .

This pattern was also obvious when relative N_{area} (N_{area} divided by N_{area} at the bottom of the crown for each tree) was plotted against relative $\delta^{13}\text{C}$ (Fig. 5). Using a mixed-effects model for the PREF01 dataset, we found that AIC increased from -4063 to -3937 when species was added

as a fixed effect (and RMSE did not change at all). This means that species did not differ in the relationship between relative N_{area} and relative $\delta^{13}\text{C}$.

Discussion

We found strong gradients of $\delta^{13}\text{C}$ within forest canopies, with leaves at the top of the canopy more enriched (Fig. 1). This finding is in agreement with many other studies (for a review see Broadmeadow and Griffiths 1993; Le Roux et al. 2001). The average range in $\delta^{13}\text{C}$ within crowns varied between 1.2 (western larch) and 3.8‰ (grand fir). These values are consistent with previously reported magnitude of $\delta^{13}\text{C}$ variation within crowns (Broadmeadow and Griffiths 1993), but not quite as large as the 6‰ range in beech (*Fagus sylvatica*) found by Schleser (1990).

Leaf $\delta^{13}\text{C}$ and N_{area} were highly correlated within crowns of the studied conifer species. The same was true for $\delta^{13}\text{C}$ and LMA, because N_{mass} is relatively constant within conifer canopies (e.g., Niinemets 1997a). Many other studies have documented this relationship, regardless of whether the LMA or N_{area} samples came from within canopies (Hanba et al. 1997; Niinemets et al. 1999; Roberts et al. 1999; Turney et al. 2002), across altitude gradients

Table 1 Statistics of the fit between $\delta^{13}\text{C}$ and several different predictor variables, for each species separately

Species	N_{area}			LMA			N_{mass}			Canopy depth			Height		
	R^2	p -value	CV b_1	R^2	p -value	CV b_1	R^2	p -value	CV b_1	R^2	p -value	CV b_1	R^2	p -value	CV b_1
Grand fir	0.858	0	9.8	0.884	0	14.3	0.447	0	11.4	0.795	0	26.443	0.853	0	41.8
Subalpine fir	0.649	0	8.729	0.718	0	3.9	0.288	0.997	37044.4	0.643	0	0.396	0.623	0	28.6
Western larch	0.289	0.002	49.779	0.583	0	37.5	0.411	0.002	22.4	0.349	0.034	18.53	0.388	0.07	45.8
Lodgepole pine	0.485	0	30.741	0.509	0	63.8	0.476	0.925	2326.1	0.516	0.01	54.496	0.507	0.009	74.8
Douglas-fir	0.666	0	13.605	0.619	0	26.9	0.4	0	28.2	0.607	0	31.035	0.638	0	45.2
Western red-cedar	0.78	0	9.536	0.682	0	20.4	0.452	0	68.5	0.622	0	24.545	0.61	0	42.7
Western hemlock	0.627	0	16.162	0.668	0	4.2	0.363	0.018	42.1	0.663	0	23.025	0.652	0	20.2

Note. The predictor variable was always log-transformed. R^2 measures the proportion of variation explained, the p -value is from the mixed-effects model. CV b_1 is the coefficient of variation of the slope random effect (%). Species with less than 10 trees in the dataset (ponderosa pine, western white pine) were excluded from this analysis.

(Vitousek et al. 1990; Sparks and Ehleringer 1997; Cordell et al. 1999; Hultine and Marshall 2000; Li et al. 2004), or sites with different water stress (Lamont et al. 2002; Samuelson et al. 2003; MacFarlane et al. 2004). The relationship between N_{area} and $\delta^{13}\text{C}$ was not linear; $\delta^{13}\text{C}$ approached an asymptote when N_{area} was large (Figs. 2 and 4). This effect was especially obvious for western hemlock, western red-cedar, and grand fir, the three most shade-tolerant species in the sample. Although many studies have reported correlations between N_{area} (or LMA) and $\delta^{13}\text{C}$, none has previously described the nonlinearity of the relationship, probably because of limited sample variation and sample size.

Many studies have attributed variation in $\delta^{13}\text{C}$ within canopies to variation in irradiance flux (Zimmermann and Ehleringer 1990; Berry et al. 1997; Le Roux et al. 2001). Indeed, the irradiance flux changes dramatically from top to the bottom in the canopy (e.g., Parker et al. 2002), but it is not clear whether irradiance has a direct or an indirect effect on $\delta^{13}\text{C}$.

Some authors have attempted to explain this relationship with irradiance flux as a consequence of the correlation between $\delta^{13}\text{C}$ and c_i . However, there is an abundance of experimental evidence for the constancy of c_i with PPFD for a given leaf, except at low PPFD (Wong and Farquhar 1979; Sharkey and Raschke 1981; Meinzer 1982; Farquhar and Wong 1984; Wong et al. 1985; Leuning 1995). Le Roux et al. (2001) did simulate a strong decrease of c_i with irradiance, but their simulation was based on a rather empirical stomatal conductance model (see Niinemets et al. 2004a).

The constancy of c_i with PPFD implies that some other cause of the canopy variation in $\delta^{13}\text{C}$ must be found. One possibility is variation in stomatal conductance. However, stomatal conductance often varies in proportion to assimilation rate, both for a given leaf (Teskey et al. 1986; Leuning 1995) and across leaves with different photosynthetic capacity (Körner et al. 1979; Wong and Farquhar 1979; Farquhar and Wong 1984; Schulze et al. 1994; Cernusak and Marshall 2001). This proportionality is also assumed by different models of gas exchange (summarized by Katul et al. 2000). Several studies have shown an increase in g_s toward the top of the canopy in conifer species, although they appear to be rather weak (Loustau and Moussa 1989; Brooks et al. 1997), or not to occur at all (Watts et al. 1976; Beadle et al. 1985; Sellin and Kupper 2004). In contrast, Livingston et al. (1998) attributed the gradient in $\delta^{13}\text{C}$ to a steeper increase in A than g_s , which would decrease c_i toward the top of the canopy.

On the other hand, $\delta^{13}\text{C}$ has been shown to be a rather strong function of irradiance for a single leaf (von Caemmerer and Evans 1991; Broadmeadow and Griffiths 1993; Gillon et al. 1998; Evans and Loreto 2000), showing that $\delta^{13}\text{C}$ is not solely a function of c_i . In recent years, the importance of the conductance from intercellular spaces to the chloroplast (transfer conductance, g_i) has become apparent (Evans et al. 1986; Evans and Loreto 2000). This transfer conductance would increase $\delta^{13}\text{C}$ with increasing irradiance, even with constant c_i (von Caemmerer and

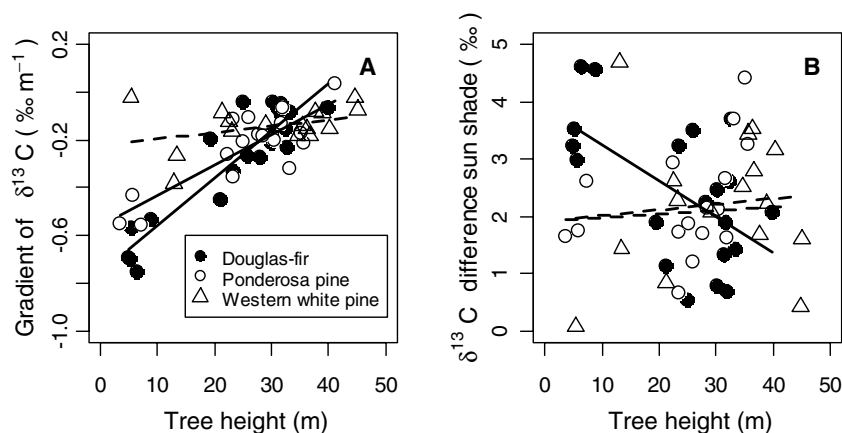


Fig. 3 **a** Difference in $\delta^{13}\text{C}$ between top and bottom canopy leaves for Douglas-fir, ponderosa pine, and western white pine. Regression was only significant for Douglas-fir ($p = 0.006$, $R^2 = 0.29$). **b** Vertical gradients of $\delta^{13}\text{C}$ for the same species as a function of total

tree height. Each gradient was estimated with linear regression for each tree. The overall relationship was significant for the data combined ($p < 0.001$), and significant for Douglas-fir and Ponderosa pine ($p < 0.001$), but not for western white pine ($p = 0.16$)

Evans 1991; Broadmeadow and Griffiths 1993; Gillon et al. 1998; Evans and Loreto 2000). A simple model of the concentrations of CO_2 in air, intercellular spaces, and chloroplast can be written as (Hanba et al. 2003)

$$c_c = c_i - \frac{A}{g_i}, \quad (2)$$

where c_c is the CO_2 concentration in the chloroplast ($\mu\text{mol mol}^{-1}$), c_a the ambient CO_2 concentration ($\mu\text{mol mol}^{-1}$), A the assimilation rate of the leaf ($\mu\text{mol m}^{-2} \text{s}^{-1}$), g_s is stomatal conductance ($\text{mol m}^{-2} \text{s}^{-1}$), and g_i is transfer conductance ($\text{mol m}^{-2} \text{s}^{-1}$). This equation shows that if everything else is equal, the difference between c_c and c_i is determined by the assimilation rate (e.g., Gillon et al. 1998). Le Roux et al. (2001) assumed g_i to be proportional to A within the canopy, in which case g_i has little to no effect on the gradient in c_c (Niinemets et al. 2004a). This proportionality was assumed based on reviews by Loreto and Evans (2000), who showed that g_i is roughly proportional to assimilation capacity across species. However, for a Douglas-fir canopy, Warren et al. (2003) found no difference in g_i between sun and shade leaves. Photosynthetic capacity was however quite different, as shown by difference in N_{area} . If g_i is nearly constant, variation in c_c occurs when A varies, for example with irradiance or photosynthetic capacity (Eq. (2); Farquhar et al. 1989; von

Caemmerer and Evans 1991; Kogami et al. 2001). Transfer conductance could thus explain the correlation between $\delta^{13}\text{C}$ and N_{area} , despite constant c_i , in vertical canopy gradients.

The studied conifer species showed quite different $\delta^{13}\text{C}$ values given N_{area} (Fig. 4), but the increase per unit N_{area} was remarkably similar (Table 2). Also, when N_{area} and $\delta^{13}\text{C}$ were expressed relative to their values at the bottom of the crown, there were no species effects at all (Fig. 5). Suppose stomatal conductance is strictly proportional to assimilation rate (A), so that c_i is constant. It then follows that *relative* changes in $\delta^{13}\text{C}$ are associated only with $c_i - c_c$ (Eq. (2)), which is a direct function of assimilation rate. Because photosynthetic capacity scales with N_{area} similarly for most conifer species (Duursma et al. 2005), this would mean a similar increase in A per unit N_{area} , and hence a similar increase in $\delta^{13}\text{C}$ if transfer conductance is similar among species. Unfortunately, for this last assertion there is no data available at present (see Warren et al. 2003). In fact, $\delta^{13}\text{C}$ increases nonlinearly with N_{area} (Fig. 4), although photosynthetic capacity scales linearly with N_{area} , which cannot be explained by this simple model.

In our dataset, species with higher shade tolerance (western red-cedar, western hemlock, and grand-fir) showed higher variation in N_{area} and $\delta^{13}\text{C}$ than species with low shade tolerance (western larch, lodgepole pine, ponderosa pine) (Fig. 2). Other studies have described smaller ranges in N_{area} or related variables in shade-intolerant compared to shade-tolerant species (Niinemets 1997b; Bond et al. 1999), but this has not been extended to $\delta^{13}\text{C}$. Because the slope of $\delta^{13}\text{C}$ with N_{area} is so constant between our species, more variation in N_{area} within the canopy would directly imply more variation in $\delta^{13}\text{C}$. It remains to be seen whether this mechanism explains the differences in within-canopy variation among species and sites (review by Broadmeadow and Griffiths 1993).

Recently, Niinemets et al. (2004a) showed that sun leaves at the top of an aspen (*Populus tremula*) canopy closed stomata earlier during the day and longer than shade leaves

Table 2 Statistics for the mixed-model fits of the form $\delta^{13}\text{C} = (\beta_0 + b_1) + (\beta_1 + b_1) \log(N_{\text{area}})$

	df	AIC	RMSE
Full model	20	2200.8	0.944
Species intercept	12	2221.3	0.946
No species	4	2425.6	1.512

Note. Species effect was added to intercept and slope (Full model), only to the intercept or not at all. df: degrees of freedom; AIC: Akaike's Information Criterion (lower is better); RMSE: root mean squared error (units of ‰).

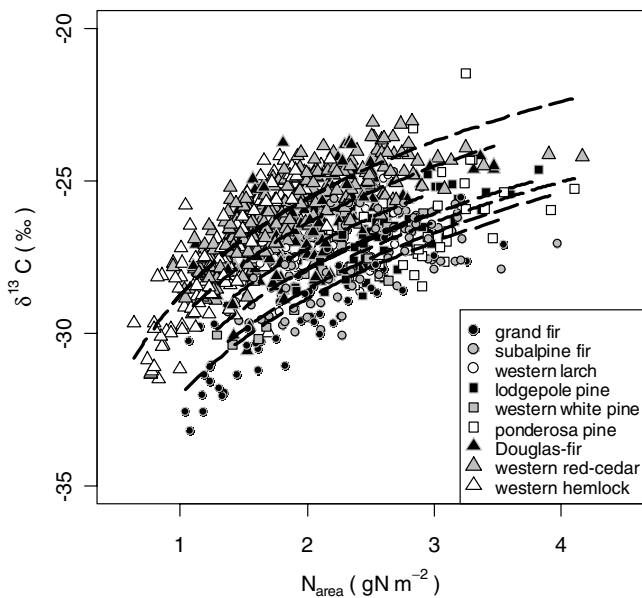


Fig. 4 The relationship between $\delta^{13}\text{C}$ and N_{area} for all nine conifer species. The lines drawn are estimated with the mixed-effects model, with $\log(N_{\text{area}})$ as the independent variable. The intercept is different for each species, but only one slope was fitted

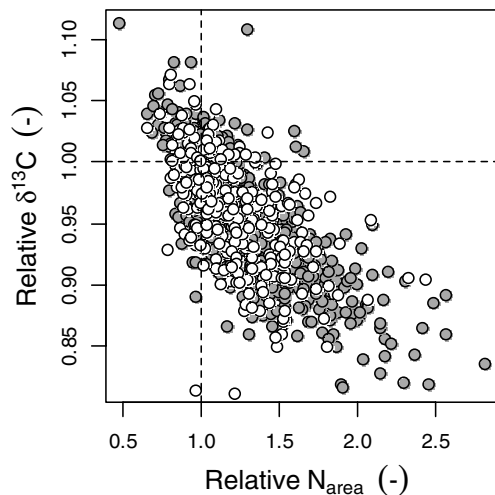


Fig. 5 N_{area} and $\delta^{13}\text{C}$ relative to their values at the bottom of the crown for each tree. All data for all species are shown. White circles: PREF94 dataset, grey circles: PREF01 dataset

low in the canopy. This differential stomatal closure within canopies was held responsible for observed gradients in c_i , and hence $\delta^{13}\text{C}$. Niinemets et al. (2004a) speculated that leaves high in the canopy were more drought-stressed than leaves low in the canopy. If hydraulic limitation (Ryan and Yoder 1997) were the main mechanism controlling height-related differences in stomatal conductance, large trees would have a larger difference in c_i between sun and shade leaves because the path length for water transport to sun leaves is much longer. The vertical gradient in c_i (decrease per meter) should be similar, however, because hydraulic limitation is related to gravitational potential (Ryan and Yoder 1997). Our results contrast with this expectation

(Fig. 3). We found that small open-grown trees showed a steeper vertical gradient in $\delta^{13}\text{C}$ than did large, mature trees, but that the difference in $\delta^{13}\text{C}$ between sun and shade leaves was unrelated to tree size (except for Douglas-fir where the opposite pattern was found). Livingston et al. (1998) found a steep gradient in $\delta^{13}\text{C}$ within one very short *Pinus radiata* tree, also unlikely to be explained by hydraulic limitation. On very tall trees (ca. 120 m) Koch et al. (2004) demonstrated that foliage was water limited and related this to patterns in $\delta^{13}\text{C}$. In more modestly sized trees, several studies have provided evidence that shade leaves are more sensitive to drought than sun leaves, at least in some species. Lemoine et al. (2002) found larger sensitivity of shade foliage to drought than sun foliage within a *F. sylvatica* crown. Dang et al. (1997) reported higher sensitivity to vapor pressure deficit in shade leaves than sun leaves in *Pinus banksiana* and *Populus tremuloides*, and Sellin and Kupper (2004) found the same for *Picea abies*. Bonal et al. (2000) found larger sensitivity to drought in shade leaves than sun leaves in some of their tropical rainforest species, although the reverse was true in other species. We do not disagree with the findings of Niinemets et al. (2004a), but it seems unlikely that differential drought stress within the canopy is the main factor causing $\delta^{13}\text{C}$ variation in the forests we describe here.

Several studies have pointed out the importance of variation in hydraulic conductance within crowns as a source of variation in leaf $\delta^{13}\text{C}$, which might be a reason for the patterns observed by Niinemets et al. (2004a). Waring and Silvester (1994) and Walcroft et al. (1996) found that $\delta^{13}\text{C}$ was more negative in long branches, which have lower hydraulic conductance, which in turn causes a larger constraint on stomatal conductance (see e.g., Cernusak and Marshall 2001). Although this trend is probably important to study variation between branches of similar exposure (Walcroft et al. 1996), this mechanism does not help to explain variation within crowns of forest trees, since longer branches occur lower in the crown, but foliage on those branches exhibits more discrimination against ^{13}C . For differential hydraulic conductance (k_L) within crowns to explain observed $\delta^{13}\text{C}$ gradients, sun leaves at the top of the canopy must have lower k_L than shade leaves at the bottom, although often the opposite is found in conifers. Lemoine et al. (2002) reported that sun branches had higher k_L and higher resistance to cavitation, results that are supported by Cochard et al. (1999) for the same species, and Sellin and Kupper (2004) for *P. abies*. Mencuccini and Grace (1996) found lower whole-branch hydraulic conductivity of shade branches in *Pinus sylvestris*, and Hubbard et al. (2002) found no difference in k_L between bottom canopy and top canopy branches in *P. ponderosa*. The evidence for within-tree variation in k_L does not generally support higher drought sensitivity of sun leaves.

$\delta^{13}\text{C}$ has been used extensively as an integrative indicator of water-use efficiency (cf. Farquhar and Richards 1984; Farquhar et al. 1989), and soil water availability (cf. Stewart et al. 1995). Although it has been noted that this is dangerous practice when other factors vary (Warren et al. 2001), many studies interpret $\delta^{13}\text{C}$ as if the sole driver were water-

stress induced reduction in stomatal conductance. Because $\delta^{13}\text{C}$ is largely determined by the balance between supply (stomatal conductance) and demand (assimilation rate), inferences about conductance require that the demand side be accounted for. This study shows that a significant proportion of variation in $\delta^{13}\text{C}$ can be explained with variation in photosynthetic capacity. For a group of co-occurring temperate conifer species, the slope of $\delta^{13}\text{C}$ with N_{area} was not different, implying that within-crown variation of $\delta^{13}\text{C}$ is related to the variation in photosynthetic capacity. The most likely explanation for this correlation as well as vertical canopy gradients in $\delta^{13}\text{C}$ is a transfer conductance that causes a drawdown of $[\text{CO}_2]$ in the chloroplast with increasing assimilation rate. The implication is that, at least in this group of conifer species, photosynthetic capacity (as indicated by N_{area}) must be used to explain variation in $\delta^{13}\text{C}$, both within the canopy and among species.

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