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Altitude trends in conifer leaf morphology and stable carbon isotope composition

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Abstract The natural ratio of stable carbon isotopes ($\delta^{13}\text{C}$) was compared to leaf structural and chemical characteristics in evergreen conifers in the north-central Rockies, United States. We sought a general model that would explain variation in $\delta^{13}\text{C}$ across altitudinal gradients. Because variation in $\delta^{13}\text{C}$ is attributed to the shifts between supply and demand for carbon dioxide within the leaf, we measured structural and chemical variables related to supply and demand. We measured stomatal density, which is related to CO_2 supply to the chloroplasts, and leaf nitrogen content, which is related to CO_2 demand. Leaf mass per area was measured as an intermediate between supply and demand. Models were tested on four evergreen conifers: *Pseudotsuga menziesii*, *Abies lasiocarpa*, *Picea engelmannii*, and *Pinus contorta*, which were sampled across 1800 m of altitude. We found significant variation among species in the rate of $\delta^{13}\text{C}$ increase with altitude, ranging from 0.91‰ km^{-1} for *A. lasiocarpa* to 2.68‰ km^{-1} for *Pinus contorta*. Leaf structure and chemistry also varied with altitude: stomatal density decreased, leaf mass per area increased, but leaf nitrogen content (per unit area) was constant. The regressions on altitude were particularly robust in *Pinus contorta*. Variables were derived to describe the balance between supply and demand; these variables were stomata per gram of nitrogen and stomata per gram of leaf mass. Both derived variables should be positively related to internal CO_2 supply and thus negatively related to $\delta^{13}\text{C}$. As expected, both derived variables were negatively correlated with $\delta^{13}\text{C}$. In fact, the regression on stomatal density per gram was the best fit in the study ($r^2=0.72$, $P<0.0001$); however, the relationships were

species specific. The only general relationship observed was between $\delta^{13}\text{C}$ and LMA: $\delta^{13}\text{C}$ (‰) = $-32.972 + 0.0173 \times \text{LMA}$ ($r^2=0.45$, $P<0.0001$). We conclude that species specificity of the isotopic shift indicates that evergreen conifers demonstrate varying degrees of functional plasticity across environmental gradients, while the observed convergence of $\delta^{13}\text{C}$ with LMA suggests that internal resistance may be the key to understanding inter-specific isotopic variation across altitude.

Key words Carbon isotope ratio · Stomatal density · Leaf nitrogen content · Leaf mass per area · Evergreen conifers

Introduction

The relation between stable carbon isotope ratio and photosynthetic water-use efficiency has led to widespread use of isotopic analyses in plant physiological ecology. Stable carbon isotope composition, expressed as $\delta^{13}\text{C}$, reflects the balance between mesophyll demand for carbon dioxide (A) against the diffusive supply through the stomata (g). More precisely, $\delta^{13}\text{C}$ is associated with the ratio of CO_2 partial pressure within the leaves (P_{int}) to the CO_2 partial pressure outside the leaves (P_{amb}) (Farquhar et al. 1989). The significance of isotopic variation lies in its broad correlation with photosynthetic water-use efficiency (WUE), defined as the molar ratio of photosynthetic carbon gain to transpirational water loss (Farquhar and Sharkey 1982). Considerable effort has gone into the description of sources of variation in $\delta^{13}\text{C}$, which varies among co-occurring species (DeLucia et al. 1988; Gower and Richards 1990; Marshall and Zhang 1994), genotypes within species (Zhang et al. 1993), and along environmental gradients (DeLucia and Schlesinger 1991; Lajtha and Getz 1993; Panek and Waring 1995, 1997).

Shifts in $\delta^{13}\text{C}$ at the intraspecific level are consistently observed over altitudinal gradients (Körner et al. 1988, 1991; Vitousek et al. 1990; Marshall and Zhang

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1994; Sparks and Ehleringer 1997); however, the mechanisms underlying this trend are unknown. Ecologists have reported correlations of $\delta^{13}\text{C}$ with various abiotic factors over altitude gradients. These factors include soil moisture (Beerling et al. 1996; Sun et al. 1996), air temperature (Panek and Waring 1995), atmospheric CO_2 concentrations (Ehleringer and Cerling 1995; Marshall and Monsrud 1996), and barometric pressure (Marshall and Zhang 1994). The $\delta^{13}\text{C}$ trend has also been correlated with leaf morphological and physiological traits that vary with altitude. Correlated leaf traits include leaf thickness (Vitousek et al. 1990; Cordell et al. 1998), leaf nitrogen content (Morecroft and Woodward 1996), stomatal conductance (Meinzer et al. 1992), and stomatal density (number of pores per leaf surface area) (Körner et al. 1989).

Altitudinal patterns of stomatal density (SD) are particularly relevant to the $\delta^{13}\text{C}$ trend. Stomatal density generally increases with altitude, and presumably increases g (Körner and Cochrane 1985; Woodward and Bazzaz 1988). Stomatal conductance increases the supply of CO_2 to the interior of the leaf and would be expected to reduce $\delta^{13}\text{C}$ of fixed carbon. Increases in SD (and g) should reduce, rather than enhance $\delta^{13}\text{C}$ composition of leaves with altitude. This discrepancy leads to the suggestion that the variation in SD is not the cause of the observed increase in $\delta^{13}\text{C}$ with altitude, but is instead a response to some other stimulus.

Correlations between $\delta^{13}\text{C}$, leaf nitrogen content (N), and leaf thickness might also be expected across altitude gradients. Photosynthetic capacity (A_{max}) generally increases with leaf nitrogen content (per unit area) because photosynthetic enzymes such as RuBP carboxylase contain large quantities of N (Field 1983; Field and Mooney 1986; Friend and Woodward 1990). Levels of N in plant tissues have been positively correlated with altitude, and may increase CO_2 demand at the sites of carboxylation (Körner 1989; Sparks and Ehleringer 1997). This increase might be global, particularly if low temperatures and short growing seasons restrict shoot growth more than they restrict N uptake by roots, concentrating leaf N. Likewise, leaf mass per area (g m^{-2}) increases with altitude (Woodward 1986; Williams et al. 1995), and is often correlated with $\delta^{13}\text{C}$ (Vitousek et al. 1990; Körner et al. 1991). One mechanism that would link leaf mass per area (LMA) with $\delta^{13}\text{C}$ is that thicker leaves contain a higher quantity of photosynthetic enzymes, and hence have greater demand for carbon dioxide, per unit area (Körner and Diemer 1987). Alternatively, the increase in $\delta^{13}\text{C}$ may be caused by an increase in the length of the internal diffusion pathway from the stomata to the chloroplasts, which would reduce carbon dioxide supply at the site of carboxylation (Evans et al. 1986; Vitousek et al. 1990).

If the above structural and physiological characteristics describe the balance between supply and demand of CO_2 , then a combination of these characteristics might be expected to provide the best correlation with stable carbon isotope ratios. SD should serve as a surrogate for

CO_2 supply (g), while N (per unit leaf area) should serve as a surrogate for CO_2 demand (A). LMA serves as an intermediate depending on the mechanism by which it controls the balance between supply and demand; i.e., A_{max} , length of diffusion pathway, or both. Combining these variables into derived variables should provide simultaneous estimates of the balance point between supply and demand. Here we test correlations between $\delta^{13}\text{C}$ and two derived variables, number of stomata per gram of nitrogen (St/N) and number of stomata per leaf mass (St/lm).

In this study, we report trends in leaf $\delta^{13}\text{C}$, structure, and chemistry in four conifer species that occur across a broad altitudinal range in the north-central Rockies. Our goal was to determine whether some structural or physiological trait would yield a generalizable correlation over altitude. We tested the following hypotheses: (1) that carbon isotope ratios in the needles of these species shift consistently over separate altitudinal transects, and (2) that shifts in isotope ratios are correlated with leaf structural and physiological traits that are surrogates of CO_2 supply and demand; these traits included stomatal density, leaf nitrogen content, leaf mass per area, stomata per leaf nitrogen, and stomata per leaf mass.

Materials and methods

During the summer of 1996, mature *Pseudotsuga menziesii* and *Pinus contorta* stands were sampled along a transect in southern Idaho, while mature stands of *Abies lasiocarpa* and *Picea engelmannii* were sampled along a transect in northern Idaho and western Montana (Table 1). To maintain a constant ambient CO_2 concentration and $\delta^{13}\text{C}$ throughout the gradients, we generally sampled from stands with <30% canopy cover. Previous-year (1995) needles were collected from the south side of open crowns using a 4-m pruning pole. To minimize the effects of stem hydraulic conductivity limitations on carbon isotope discrimination, (Waring and Silvester 1994) we sampled from the same approximate height (7 m) and branch length. Six to eight trees were sampled in each stand.

Stomatal counts were performed on two needles from each sample tree. Stomatal densities were determined using a dissecting microscope equipped with a scaled grid. All counts were conducted near the middle of the needle to avoid variation that might occur at the base and at the tip. The stomatal density (number of stomata per surface needle area) was determined as:

$$SD = \frac{SC}{W_n \cdot L_g} \quad (1)$$

where SC is the stomatal count, W_n is the needle width (mm), and L_g is the length (mm) of the grid measured through the microscope. Because three of the four species analyzed are amphistomatous (stomata on both upper and lower surfaces) stomatal densities for all species are presented as the average of the upper and lower surfaces.

For each sample tree, projected leaf area was determined on 12–20 needles Sigma Scan, v.4 for Windows. (Jandel Scientific, Modesto, Calif., USA), calibrated to a wire rod similar in size and shape to conifer needles. Needles were oven-dried for 48 h at 70°C and weighed to determine LMA. Because we had no correction factor to account for the three-dimensional shape of conifer needles across the gradient, we did not attempt to estimate all-sided leaf area. A mortar and pestle was used to grind the needles into fine powder and each sample was split into two subsets. Nitrogen content per unit mass (N_{mass}) was determined on one subset

Table 1 Altitude, latitude, longitude, slope, and aspect of tree stands sampled along two transects in the north-central Rockies

Site no.	Altitude (m)	Latitude	Longitude	Mean slope (%)	Aspect
<i>Pseudotsuga menziesii</i>					
1	2560	44°12'00"	114°46'30"	31–40%	221
2	2256	44°13'30"	114°47'30"	23–27%	196
3	1829	44°16'00"	114°45'00"	30–35%	194
4	1402	44°08'00"	115°18'30"	15–17%	268
5	945	44°03'00"	116°56'30"	3%	4
<i>Abies lasiocarpa</i>					
1	2280	47°40'00"	114°12'30"	5–10%	340
2	1829	47°01'00"	116°01'30"	5–10%	160
3	1515	47°02'00"	116°06'30"	5%	190
4	1067	46°45'30"	116°08'00"	15–18%	246
5	808	46°45'00"	116°56'30"	0%	n/a
<i>Picea engelmannii</i>					
1	2280	47°39'00"	114°11'30"	5–10%	340
2	1792	46°34'30"	114°37'00"	43–45%	318
3	1515	47°00'30"	116°40'30"	24%	158
4	1189	46°34'30"	114°37'00"	12%	120
5	841	47°00'30"	116°40'30"	0%	n/a
<i>Pinus contorta</i>					
1	2591	44°11'30"	114°46'30"	5–10%	350
2	2256	44°13'30"	114°47'30"	10–15%	170
3	1875	44°15'00"	114°52'00"	0–5%	90
4	1417	44°08'30"	115°18'00"	0%	n/a
5	1158	44°05'00"	115°36'30"	0%	n/a

(60–100 mg per sample) with a Leco 1000 CHN analyzer (Leco Corporation, St Joseph, Mich., USA). Results were calibrated against a wide range of organic and inorganic samples. The other subset was analyzed for $\delta^{13}\text{C}$ with a continuous-flow ratioing mass spectrometer (Europa Scientific Ltd., Cheshire, UK) at the University of Waikato, New Zealand. All isotope samples were run against a standard and expressed in relation to the Pee Dee Belemnite standard.

The ambient CO_2 partial pressure (P_{amb}) was estimated by multiplying a constant mole fraction, $360.9 \mu\text{mol mol}^{-1}$ (C.D. Keeling, personal communication) of CO_2 by total barometric pressure P_B (pascals), estimated according to Jones (1983):

$$P_B = \frac{101.325}{e^{[(z/29.3)/T_k]}} \quad (2)$$

where z is altitude above sea level (m) and T_k is air temperature (K). T_k was estimated from average maximum and minimum summertime (May–August) temperatures. Weather data were compiled from weather stations nearest each transect where relative humidity was available. Southern transect data were compiled from Boise, Idaho (2838 m above mean sea level), while northern transect data were from Lewiston, Idaho (1413 m above mean sea level). Maximum and minimum temperatures were corrected for altitude using a lapse rate of $-6.4 T_k \text{ km}^{-1}$ (Finklin 1986) down to dewpoint. Data presented by Finklin (1986) suggest that minimum temperatures in regions below 900 m altitude remain above dewpoint. However, because stands sampled below 900 m occurred in cold air drainages, we assumed minimum temperatures both above and below 900 m were constrained to dew point temperatures. Mean summer needle temperature was assumed to equal mean summertime air temperature. P_{int} was derived algebraically using the carbon isotope data and Eq. 2 (Farquhar et al. 1982).

Regression analysis was performed to determine the mean response of each species against altitude, followed by regression analysis to relate carbon isotope discrimination to other physiological and structural traits. Species differences were analyzed using analysis of covariance. JMP 3.15 (SAS Institute Inc.) was used for all statistical analyses, with $P \leq 0.10$ as the significance level.

Results

Carbon isotope ratios ($\delta^{13}\text{C}$) increased linearly with altitude in all species ($P < 0.0001$, Fig. 1), but slopes varied among species ($P < 0.0001$). Slopes ranged from 0.91‰/km in *Abies* to 2.68‰/km in *Pinus*. The mean rate of increase was 1.57‰/km . We compared species by estimating $\delta^{13}\text{C}$ values at 1500 m ($\delta^{13}\text{C}_{1500}$), which is near the middle of the sampled gradients. At this altitude, *Pseudotsuga* was least negative (-25.70‰), followed by *Picea* (-26.13‰), *Abies* (-27.30‰), and *Pinus* (-27.44‰) (Table 2). We estimated the CO_2 partial pressure gradient into the leaf ($P_{\text{amb}} - P_{\text{int}}$) using the isotope data and modeled total barometric pressure (Eq. 2). $P_{\text{amb}} - P_{\text{int}}$ was independent of altitude in all species except *Pinus*, for which the partial pressure gradient decreased by $2.30 \times 10^{-3} \text{ Pa m}^{-1}$ ($P < 0.0001$, Fig. 2).

Leaf structure and chemistry also varied with altitude, but the patterns varied among species; *Pinus* and *Abies* generally showed altitudinal trends, but *Pseudotsuga* and *Picea* did not. For example, the decrease in SD with altitude was clear in *Pinus* and especially in *Abies*, but SD was independent of altitude for *Pseudotsuga* and *Picea* (Table 2). Similarly, leaf mass per unit area was strongly correlated with altitude in *Pinus* and *Abies*, but not in *Pseudotsuga* or *Picea* (Table 2) and number of stomata per gram of leaf nitrogen (St/N), and the number of stomata per unit leaf mass (St/lm) both decreased with altitude in *Pinus* and *Abies*, but not in *Pseudotsuga* or *Picea* (Table 2, Fig. 3). Conversely, nitrogen content (N_{mass} , g g^{-1}) decreased in all species (Table 3). Leaf nitrogen content per unit area (N_{area} , g m^{-2}) did not change across

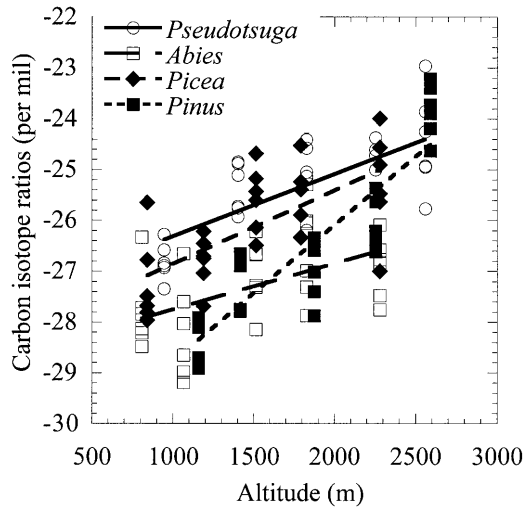


Fig. 1 Stable carbon isotope ratios ($\delta^{13}\text{C}$) of conifer needles measured along an altitude gradient in the north-central Rockies. Individual regression equations are presented in Table 2, analysis of covariance is presented in Table 3

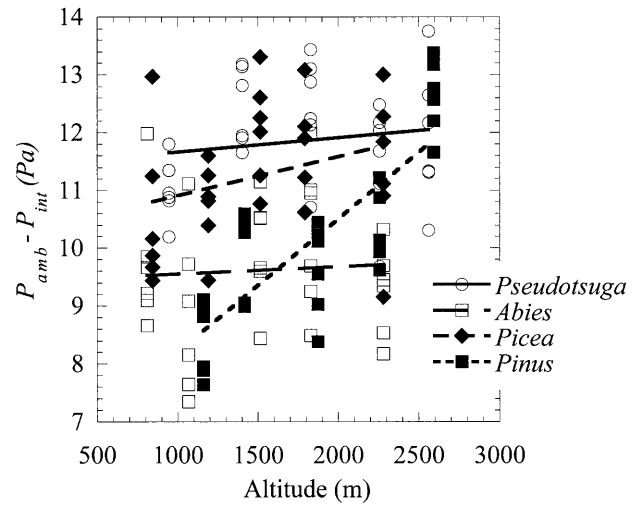


Fig. 2 Difference in CO_2 partial pressure between the ambient atmosphere (P_{amb}) and the intercellular air spaces (P_{int}) within the conifer needles predicted from isotope values and modeled total pressure. Individual regression equations are presented in Table 2, analysis of covariance is presented in Table 3

Table 2 Regression equations of individual species' structural and physiological leaf traits across altitude. Mean response of each variable was compared by correcting to a common altitude (1500 m) ($\delta^{13}\text{C}$ ratio of ^{13}C to ^{12}C , P_{amb} CO_2 partial pressure outside the

leaves, P_{int} CO_2 partial pressure within the leaves, SD stomatal density, N leaf nitrogen content, LMA leaf mass per area, St/N number of stomata per gram of leaf nitrogen, St/lm number of stomata per leaf mass)

Species	Statistic	$\delta^{13}\text{C}$	$P_{\text{amb}}-P_{\text{int}}$	SD (St mm^{-2})	N_{mass} (g g^{-1})	N_{area} (g m^{-2})	LMA (g m^{-2})	St/N	St/lm
<i>Pseudotsuga</i>	Intercept	-27.545	11.416	52.58	0.00937	3.678	392.24	1.47×10^7	133126
	Slope	0.00123* ⁴	0.00025	-0.00034	-649×10^{-7} * ¹	-0.00016	0.01265	501.70	-4.13
	Mean at 1500 m	-25.700	11.791	52.07	0.00840	3.438	411.22	1.55×10^7	126928
<i>Abies</i>	Intercept	-28.666	9.243	71.08	0.01026	3.646	330.75	2.00×10^7	236551
	Slope	0.00091* ²	0.00084	-0.01057* ²	-6.95×10^{-7} * ²	-0.00002	0.03175* ²	-3006.49* ¹	-49.94* ⁴
	Mean at 1500 m	-27.301	10.503	55.24	0.00922	3.616	378.38	155×10^7	161638
<i>Picea</i>	Intercept	-28.305	10.228	34.61	0.00964	3.714	377.76	1.02×10^7	95003
	Slope	0.00145* ⁴	0.00068	-0.00166	-6.61×10^{-7}	-0.00018	0.01402	-273.49	-8.62
	Mean at 1500 m	-26.130	11.248	34.12	0.00865	3.444	398.79	9.79×10^6	82066
<i>Pinus</i>	Intercept	-31.464	5.903	60.86	0.00958	2.467	263.84	2.21×10^7	209149
	Slope	0.00268* ⁴	0.0023* ⁴	-0.00565* ¹	-1.95×10^{-7}	0.00055* ²	0.06213* ⁴	-3873.95* ²	-39.64* ⁴
	Mean at 1500 m	-27.444	9.359	52.39	0.00929	3.291	357.04	1.63×10^7	149687

*¹ $P < 0.1$, *² $P < 0.01$, *³ $P < 0.001$, *⁴ $P < 0.0001$

Table 3 Covariance of structural and physiological leaf traits against altitude and species ($\delta^{13}\text{C}$ ratio of ^{13}C to ^{12}C , P_{amb} CO_2 partial pressure outside the leaves, P_{int} CO_2 partial pressure within the leaves, SD stomatal density, N leaf nitrogen content, LMA leaf

mass per area, St/N number of stomata per gram of leaf nitrogen, St/lm number of stomata per leaf mass). P-values < 0.10 are in bold print

	$\delta^{13}\text{C}$	$P_{\text{amb}}-P_{\text{int}}$	SD (St mm^{-2})	N_{mass} (g g^{-1})	N_{area} (g m^{-2})	LMA (g m^{-2})	St/N	St/lm
Altitude	<0.0001	<0.0001	0.0004	0.0021	0.59	<0.0001	0.0024	<0.0001
Species	<0.0001	<0.0001	<0.0001	0.0084	0.0146	<0.0001	<0.0001	<0.0001
Altitude×Species	<0.0001	0.0002	0.0191	0.35	0.0088	0.0211	0.0088	<0.0001
r^2	0.69	0.52	0.65	0.38	0.12	0.45	0.46	0.72

the gradients for *Pseudotsuga*, *Abies*, or *Picea*, but increased in *Pinus*.

Leaf $\delta^{13}\text{C}$ was related to measured leaf traits, but these relationships were also species-specific (Table 4, Fig. 4). Generally, *Pinus* had the steepest slopes and the

strongest relationships among these species (Table 5). $\delta^{13}\text{C}$ decreased with stomatal density; species differences in this relationship were barely detectable (SD by species interaction significant at $P=0.043$, species effect significant at $P=0.058$, Table 4). The negative trend was

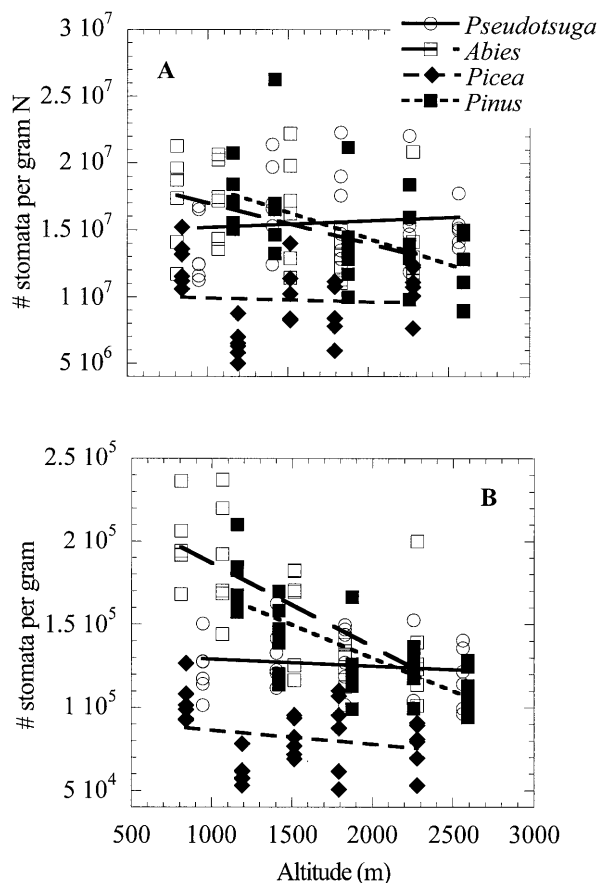


Fig. 3A,B Response of the derived variables measured across altitude. **A** The number of stomata per gram of leaf nitrogen (St/N). **B** The number of stomata per leaf mass (St/lm). Individual regression equations are presented in Table 2, analysis of covariance is presented in Table 3

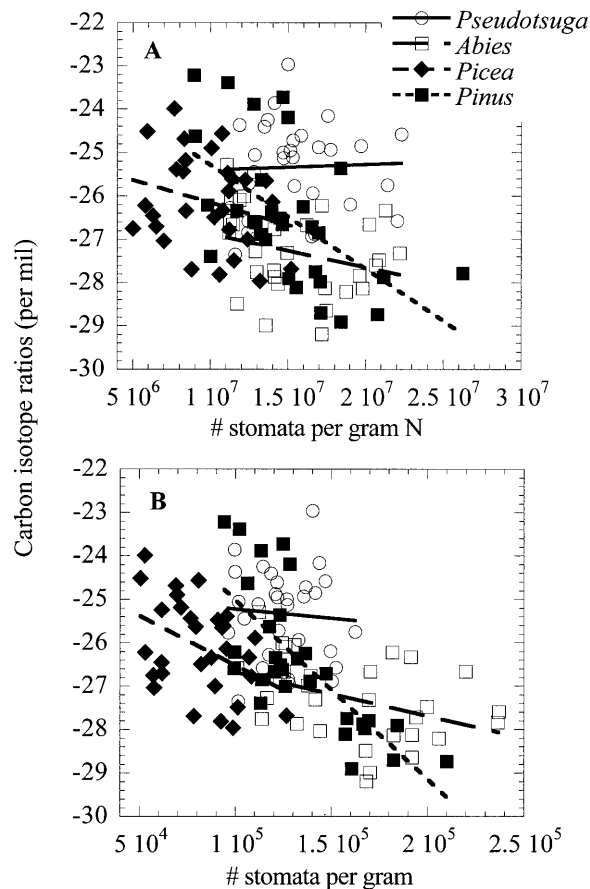


Fig. 4 Carbon isotope ratios ($\delta^{13}\text{C}$) of conifer needles as a function of **A** number of stomata per gram of leaf nitrogen (St/N), **B** number of stomata per leaf mass (St/lm). Analysis of covariance is presented in Table 4. Regressions for $\delta^{13}\text{C}$ against measured leaf traits differed among species and are presented in Table 5

Table 4 Covariance of carbon isotope ratios ($\delta^{13}\text{C}$) against leaf structural and physiological traits. (SD stomatal density, N leaf nitrogen content, LMA leaf mass per area, St/N number of stomata

per gram of leaf nitrogen, St/lm number of stomata per leaf mass). P-values <0.10 are in bold print

	SD (St mm ⁻²)	N_{mass} (g g ⁻¹)	N_{area} (g m ⁻²)	LMA (g m ⁻²)	St/N	St/lm
Leaf trait	0.0062	0.10	0.0386	<0.0001	0.0023	<0.0001
Species	0.0581	0.82	0.0094	0.25	0.0566	0.0014
Leaf trait×Species	0.0433	0.98	0.0227	0.22	0.0317	0.0011
r^2	0.36	0.28	0.36	0.45	0.39	0.49

Table 5 Regression equations of individual species' $\delta^{13}\text{C}$ values against other leaf structural and physiological traits. (SD stomatal density, N leaf nitrogen content, LMA leaf mass per area, St/N

number of stomata per gram of leaf nitrogen, St/lm number of stomata per leaf mass)

Species	Statistic	SD (St mm ⁻²)	N_{mass} (g g ⁻¹)	N_{area} (g m ⁻²)	LMA (g m ⁻²)	St/N	St/lm
<i>Pseudotsuga</i>	Intercept	-25.867	-23.703	-25.101	-31.065	-25.53	-24.814
	Slope	0.00413	-199.498	-0.069	0.01381*1	1.25×10^{-8}	-4.15×10^{-7}
<i>Abies</i>	Intercept	-25.828	-25.000	-28.206	-31.261	-26.118	-25.672
	Slope	-0.02675	-144.858	0.250	0.01281*1	-7.66×10^{-8}	-0.00001*1
<i>Picea</i>	Intercept	-24.833	-25.090	-26.581	-29.788	-25.11	-24.229
	Slope	-0.0395	-148.068	0.131	0.00924*1	-1.04×10^{-7}	-0.00002*1
<i>Pinus</i>	Intercept	-20.992	-25.708	-32.063	-33.884	-22.909	-20.878
	Slope	-0.10885*2	-83.826	1.60557*2	0.01955*4	-2.39×10^{-7} *2	-0.00004*4

*1P<0.1, *2P<0.01, *3P<0.001, *4P<0.0001

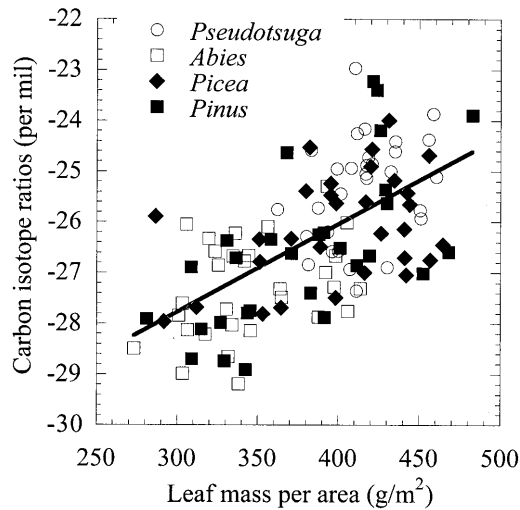


Fig. 5 Carbon isotope ratios ($\delta^{13}\text{C}$) of conifer needles as a function of leaf mass per unit area. The regression was general among species ($\delta^{13}\text{C} = -32.972 + 0.0173 \times \text{LMA}$)

most apparent in *Pinus*, in which $\delta^{13}\text{C}$ decreased by almost 0.11‰ stomata $^{-1}$ mm $^{-2}$ (Table 5). N_{mass} was unrelated to $\delta^{13}\text{C}$, but N_{area} was related in a species-specific manner, with the relationship significant only in *Pinus* ($P=0.0094$, Table 5), increasing by approximately 1.6‰ g $^{-1}$ N g $^{-1}$ tissue. The variables St/N and St/lm were also species-specific in their relations with $\delta^{13}\text{C}$ (Fig. 4A,B). Carbon isotope ratios in *Pinus* needles decreased $2.39 \times 10^{-7}\text{‰}$ per number of stomata per gram N; no decrease was measured in the other three species. Likewise, the decrease in $\delta^{13}\text{C}$ with increasing St/lm was steepest in *Pinus*, followed by *Picea*, and *Abies*, while *Pseudotsuga* $\delta^{13}\text{C}$ values were independent of St/lm (Table 5). Among these relations between leaf characteristics and stable carbon isotope ratios, only the relationship with LMA was not species-specific ($P=0.22$ for the slopes, $P=0.25$ for intercepts, Fig. 5). The percentage of variation explained by this relationship (45%) is high relative to that of the other variables. Among the variables in Table 5, only LMA is correlated with $\delta^{13}\text{C}$ in all four species. The regression equation describing this relationship across all four species is: $\delta^{13}\text{C} (\text{‰}) = -32.97 + 0.0173 \times \text{LMA}$ ($r^2=0.45$, $P<0.0001$).

Discussion

In this study, we extended the scope of common structure-function correlations that describe the balance between CO $_2$ supply and mesophyll demand by examining relations between stomatal density (SD), leaf nitrogen content (N), leaf mass per area (LMA), and leaf carbon isotope ratios ($\delta^{13}\text{C}$). We set out to determine if four co-occurring conifer species similar in life form and phylogeny would exhibit similar relationships between leaf structure and function over altitudinal gradients. We found clear relationships across the gradients, but the re-

lationships were mostly species-specific. Only the relation between leaf mass per area and $\delta^{13}\text{C}$ was general.

Several previous studies have reported a similar increase in $\delta^{13}\text{C}$ of leaves along altitudinal gradients (Körner et al. 1988, 1991; Vitousek et al. 1990; Marshall and Zhang 1994). Mean $\delta^{13}\text{C}$ values reported here are consistent with those presented by Marshall and Zhang (1994) after their discrimination values (Δ) are converted to $\delta^{13}\text{C}$ values by standard methods (Farquhar et al. 1989). Isotopic discrimination decreased ($\delta^{13}\text{C}$ increased) with altitude in all species examined by Marshall and Zhang (1994). The decrease was exactly sufficient to maintain a constant difference between P_{amb} and P_{int} , which constitutes the numerator in the equation describing photosynthetic water use efficiency (Marshall and Zhang 1994). In this study, a similar analysis predicted constant CO $_2$ partial pressure differences in all species except *Pinus*, which increased at high altitudes. Marshall and Zhang (1994) did not detect variation in *Pinus contorta*. Reasons for this discrepancy are not clear.

Previous studies have also found that the increase in $\delta^{13}\text{C}$ values with altitude was correlated with structural and physiological leaf traits. These traits include leaf conductance (Meinzer et al. 1992), leaf nitrogen content (Sparks and Ehleringer 1997), and leaf mass per area (Vitousek et al. 1990). We similarly found that $\delta^{13}\text{C}$ values were correlated with SD, N_{area} , and LMA. As far as we know, we are the first to test derived structural traits intended to describe both stomatal supply of carbon dioxide and mesophyll demand. These variables, stomata per gram of leaf nitrogen (St/N) and stomata per leaf mass (St/lm) were significantly related to $\delta^{13}\text{C}$, although the relations varied considerably among species.

Previous attempts to relate SD to altitude have obtained mixed results. Some have found that SD increases linearly with altitude (Körner and de Moraes 1979; Körner and Cochrane 1985), that it increases and then decreases again (Körner et al. 1989), or remains unchanged (Woodward 1986). Meinzer et al. (1992) speculated that $\delta^{13}\text{C}$ increases with altitude were due to decreases in g . Maximum stomatal conductance (g_{max}) presumably decreases with stomatal density (Woodward and Bazzaz 1988; van Gardingen et al. 1989), which would lead to isotopic enrichment. In this study, however, *Pinus* was the only species to demonstrate a relationship between $\delta^{13}\text{C}$ and SD. Likewise, *Pinus* had the strongest response in $\delta^{13}\text{C}$ across altitude, indicating g may have decreased with altitude. Furthermore, *Pseudotsuga*, which exhibited the highest mean $\delta^{13}\text{C}$ values, is the only species we sampled that is hypostomatous, meaning, stomata occur exclusively on the abaxial surface. Beerling and Kelly (1996) surveyed data from several studies and concluded that amphistomatous leaves have greater stomatal conductance than hypostomatous leaves. Thus, according to their conclusions, *Pseudotsuga* likely has the lowest g across the range of altitudes, yielding the highest isotope values among the species we measured. Nevertheless, the variable relationship between $\delta^{13}\text{C}$ and

SD among species suggests that SD is not the key to understanding carbon isotope trends across altitude.

Alternatively g may be constrained at higher altitudes by cool air and soil temperatures rather than low stomatal densities. Smith et al. (1984) and Kaufmann (1982) found that stomatal closure was induced by near freezing night-time air temperatures, similar to those found at high altitudes during most of the growing season. Likewise, low rhizosphere temperatures ($<7^{\circ}\text{C}$) have been shown to restrict g in *Pinus contorta* (Running and Reid 1980). The mechanism by which g is restricted is uncertain; however, Smith et al. (1984) hypothesized that cool soil and air temperatures inhibit stem sap flow, which increases the water potential gradient and induces partial stomatal closure.

Körner and Diemer (1987) suggested another potential cause for the higher $\delta^{13}\text{C}$ at high altitudes: that high-altitude plants exhibit higher photosynthetic capacities (A_{max}) relative to g . Because most leaf nitrogen is bound in photosynthetic enzymes, we hoped that N_{area} would serve as a measure of A_{max} (Evans 1989). Altitudinal trends of N_{area} varied among the four species; only *Pinus* showed a significant altitudinal increase. Likewise, $\delta^{13}\text{C}$ was correlated with N_{area} only in *Pinus*, which may explain the steeper $\delta^{13}\text{C}$ response with altitude relative to the other species. Previous studies have attributed increases in N_{area} to greater temperature sensitivity of photosynthesis relative to nitrogen mineralization (Morecroft et al. 1992). The consequent reduction in shoot biomass production concentrates foliar N, potentially increasing A_{max} . Körner and Diemer (1987) found that increases in leaf $\delta^{13}\text{C}$ in the Alps were associated with increases in leaf thickness and nitrogen per unit leaf area. Likewise, Sparks and Ehleringer (1997) discovered similar patterns over altitude in the leaves of deciduous riparian trees occurring in Utah. The lack of response in three of the four conifer species may be related to the re-distribution of leaf N post expansion. However, N_{area} is optimized when its distribution within the canopy is proportional to the distribution of intercepted photosynthetically active radiation PAR (Farquhar 1989; Stenberg et al. 1998). Accordingly, we only sampled needles that were in open canopy, and presumably were in open canopy when they expanded. We therefore suggest that there was little re-distribution of N from the time the needles were formed, and conclude that, with the exception of *Pinus*, trends in $\delta^{13}\text{C}$ do not reflect a higher A_{max} .

LMA increased with altitude and was tightly coupled with $\delta^{13}\text{C}$. However, unlike other correlations with anatomical leaf traits, this relationship was general. These results support and extend the results of Vitousek et al. (1990) from the Hawaiian tree species *Metrosideros polymorpha*. They found a significant relationship between $\delta^{13}\text{C}$ and LMA over a similar range of LMA (250–500 g m^{-2}). Vitousek et al. (1990) suggested that the longer diffusive path found in thicker leaves increases resistance and thus reduces CO_2 concentrations at the sites of carboxylation below P_{int} , the partial pressure in the substomatal cavities. Such a decrease in partial pres-

sure would tend to increase $\delta^{13}\text{C}$. Two potential alternatives should be addressed. First, projected leaf area may not accurately represent the change in all sided area of three-dimensional leaves over altitudinal gradients. B. Poulter, K.R. Hultine, and J.D. Marshall (unpublished work) investigated the relationship between LMA and leaf thickness of *Abies* and *Pinus* needles over the same transects studied here. They found that LMA was highly correlated with thickness (or change in all-sided area) for both species. LMA of *Pseudotsuga* and *Picea* was independent of altitude. We therefore conclude that LMA derived from projected leaf area is proportional to LMA derived from all-sided leaf area of these four species as well. Second, the relationship between $\delta^{13}\text{C}$ and LMA may be due to higher A_{max} (Friend and Woodward 1990; Körner and Diemer 1987). Thicker leaves generally contain more photosynthetic machinery per unit area, increasing A/g , and thus $\delta^{13}\text{C}$. However, thicker leaves must also be accompanied by higher N contents per unit leaf area for A_{max} to increase (Vitousek et al. 1990). N_{area} only increased in *Pinus*, suggesting that internal resistance, not A_{max} , is the key to $\delta^{13}\text{C}$ increases over altitude.

To what extent are the observed patterns caused by genetic variation? Of the four species studied here, only *Pseudotsuga* has undergone rigorous testing of the genetic variation in these traits. In this species, in situ variation in $\delta^{13}\text{C}$ appears to occur in spite of genetic variation that would reduce the gradient. Seedlings from high altitudes have lower $\delta^{13}\text{C}$ than seedlings from low altitudes in common-garden studies (Zhang et al. 1993). These results are particularly interesting because *Pseudotsuga* is often considered a specialist with respect to altitude; foresters recommend that the seed not be planted further than 100 m up or downslope from where it was collected (Rehfeldt 1983a, 1983b).

We hypothesized that the derived variables, St/N and St/lm would provide the best description of the trade-off between the stomatal supply of CO_2 and the mesophyll demand. We found $\delta^{13}\text{C}$ was highly correlated with both variables. In fact, stomata per leaf mass was more closely related to altitude than any variable in this study ($r^2=0.72$, Table 3). Likewise, stomata per leaf mass explained more of the variation in $\delta^{13}\text{C}$ than any other ($r^2=0.49$, Table 4). However, the relationships varied among species, and therefore could not be considered a general explanation. The lack of generality may be due to species differences in stomatal aperture (van Gardingen et al. 1989) and patchiness (Cardon et al. 1994), which would vary the supply of CO_2 independent of stomatal density. Nevertheless, because instantaneous measures of stomatal aperture and patchiness (and g) are often difficult to integrate over the course of the growing season, morphological measures such as St/N and St/lm may be useful for assessing plant function across environmental gradients.

Our data supports earlier reports of increases in $\delta^{13}\text{C}$ with altitude in evergreen conifers, but our intensive sampling has identified species differences in rate of in-

crease. *Pinus contorta* in particular demonstrated a high degree of $\delta^{13}\text{C}$ plasticity across altitude. The relationship between leaf structure and function ($\delta^{13}\text{C}$) also varied among species, indicating that plants employ multiple strategies for acquiring limited resources across environmental gradients. The single general relationship observed here was the relation between $\delta^{13}\text{C}$ and LMA, suggesting that internal resistance can to some degree limit CO_2 diffusion to sites of carboxylation in conifers. This relationship may serve to translate between leaf structure and photosynthetic function as ecosystem models are applied across species and across topographically complex terrain.

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