

Altitudinal differences in the leaf fitness of juvenile and mature alpine spruce trees (*Picea crassifolia*)

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Summary In many plant species, leaf morphology varies with altitude, an effect that has been attributed to temperature. It remains uncertain whether such a trend applies equally to juvenile and mature trees across altitudinal gradients in semi-arid mountain regions. We examined altitude-related differences in a variety of needle characteristics of juvenile (2-m tall) and mature (5-m tall) alpine spruce (*Picea crassifolia* Kom.) trees growing at altitudes between 2501 and 3450 m in the Qilian Mountains of northwest China. We found that stable carbon isotope composition ($\delta^{13}\text{C}$), area- and mass-based leaf nitrogen concentration (N_a , N_m), number of stomata per gram of nitrogen (St/N), number of stomata per unit leaf mass (St/LM), projected leaf area per 100 needles (LA) and leaf mass per unit area (LMA) varied nonlinearly with altitude for both juvenile and mature trees, with a relationship reversal point at about 3100 m. Stomatal density (SD) of juvenile trees remained unchanged with altitude, whereas SD and stomatal number per unit length (SNL) of mature spruce initially increased with altitude, but subsequently decreased. Although several measured indices were generally found to be higher in mature trees than in juvenile trees, N_m , leaf carbon concentration (C_m), leaf water concentration (LWC), St/N, LA and St/LM showed inconsistent differences between trees of different ages along the altitudinal gradient. In both juvenile and mature trees, $\delta^{13}\text{C}$ correlated significantly with LMA, N_m , N_a , SNL, St/LM and St/N. Stomatal density, LWC and LA were only significantly correlated with $\delta^{13}\text{C}$ in mature trees. These findings suggest that there are distinct ecophysiological differences between the needles of juvenile and mature trees that determine their response to changes in altitude in semi-arid mountainous regions. Variations in the fitness of forests of different ages may have important implications for modeling forest responses to changes in environmental conditions, such as predicted future temperature increases in high altitude areas associated with climate change.

Keywords: altitude, carbon isotope, ecophysiology, morphology, tree age.

Introduction

In response to ongoing increases in temperature and atmospheric CO_2 concentration (Schimel et al. 2001), tree lines may shift to higher altitudes and tree species may exhibit range expansions toward the poles (MacDonald et al. 2000, Esper and Schweingruber 2004). These shifts may further increase the atmospheric concentrations of greenhouse gases by promoting the thawing of permafrost. To model how alpine forests will adapt to predicted increases in global temperature, a detailed understanding of the ecophysiology of alpine trees is essential. Data on the responses of alpine tree species to increases in temperature and CO_2 concentrations have focused on leaf morphology and water-use efficiency by measuring stable carbon isotope ratios ($\delta^{13}\text{C}$) of leaves or bark. A strong and consistent increase in $\delta^{13}\text{C}$ has been correlated with altitude in a few species (Körner et al. 1988, 1991, Vitousek et al. 1990, Marshall and Zhang 1994, Sparks and Ehleringer 1997, Hultine and Marshall 2000, Warren et al. 2001), although a nonlinear relationship has been reported recently in other alpine trees (Qiang et al. 2003, Li et al. 2004). In addition, several analyses of single species (Cordell et al. 1998) and multiple species (Hultine and Marshall 2000, Van de Water et al. 2002) have found strong correlations between variations in altitude and both leaf characteristics and $\delta^{13}\text{C}$ values. These morphological characteristics include leaf thickness (Vitousek et al. 1990, Cordell et al. 1998), leaf nitrogen concentration (Morecroft and Woodward 1996), stomatal conductance (Meinzer et al. 1992), stomatal density (SD) (number of pores per unit leaf surface area; Körner et al. 1989) and leaf mass per unit area (LMA; Hultine and Marshall 2000). The increase in $\delta^{13}\text{C}$ with increasing altitude may also reflect changes in soil water content (Beerling et al. 1996, Sun et al. 1996), air temperature (Panek and Waring 1995), atmospheric CO_2 concentration (Ehleringer and Cerling 1995, Marshall and Monserud 1996) and barometric pressure (Marshall and Zhang 1994).

Altitudinal responses may also differ between juvenile and mature trees, because trees show a variety of ecophysiological

and anatomical changes as they grow and age (England and Attiwill 2005). Older trees tend to show a distinct decrease in leaf-specific hydraulic conductance, stomatal conductance, specific leaf area and needle length, and increases in $\delta^{13}\text{C}$, leaf thickness and LMA (Ryan and Yoder 1997, McDowell et al. 2002a, 2002b, England and Attiwill 2005). Furthermore, it has been suggested that the height of mature trees can affect internal water transport that, in turn, may limit stomatal conductance, and hence photosynthetic activity (Hubbard et al. 1999, Becker et al. 2000, Niinemets 2002, Koch et al. 2004). To date, there has been no detailed comparison of leaf characteristics in trees of different ages in high altitude regions; however, analyzing the responses of differently aged trees to increases in altitude is critical to our understanding of the potential for forests of different ages to respond to higher temperatures, changes in snow pack and increased atmospheric CO_2 concentrations. It is known that the age of forests near the tree line can vary significantly in response to climatic oscillations that have occurred over both the long and the short term, together with anthropological disturbances (Block and Treter 2001). For example, in the Asian highlands, forests at the tree line in some of the highest mountains are old, with most trees exceeding 80 years of age (Wang et al. 2004), whereas other mountain forests comprise a higher proportion of trees less than 20 years old (Wu 1980).

Picea crassifolia Kom. provides a good model for comparing leaf ecophysiological responses of differently aged trees along altitudinal gradients. This alpine spruce is mainly distributed in the Qinghai-Tibetan Plateau (QTP) at altitudes ranging from 2500 to 3500 m. On the steep northeastern slopes of the QTP, both juvenile and mature trees of this species form dominant forest stands and reach the tree line at around 3500 m. At higher altitudes, *Potentilla* shrubs replace these spruce trees. We investigated ecophysiological leaf traits in *P. crassifolia* along an altitudinal gradient on the northeast edge of the QTP. Our objectives were to determine: (1) if there are distinct differences in ecophysiological leaf traits between juvenile (2-m-tall) and mature (5-m-tall) trees at each altitudinal site; (2) whether there are consistent correlations in these traits between the two age groups; and (3) if the ecophysiological leaf traits of both juvenile and mature trees show a consistent trend in response to increases in altitude.

Materials and methods

Field sites

Needles of Qinghai spruce (*Picea crassifolia*) trees were sampled from 10 sites across a 1000 m altitudinal gradient, between 2500 and 3500 m above ordinance datum in the QTP. Weather data at three elevations representing the lower (2465 m), middle (2787 m) and higher (3483 m) areas were collected from three local meteorological stations located within the Qilian region. Mean growing season temperatures (May–September) for the lower, middle and higher elevation areas are 10.7, 10.1 and 9.56 °C respectively. The corresponding mean rainfall values are 267, 352 and 370 mm, with

evapotranspiration rates of 1080, 947 and 759 mm, respectively. These values indicate that the altitudinal gradient studied is linked to differences in temperature and variations in precipitation and evapotranspiration. The relationship between mean tree height (y) and altitude (x) can be represented as: $y = 0.00003x^2 + 0.1751x - 239.94$.

One-year-old needles were collected from the south side of the tree crowns with a pruning pole. Qinghai spruce trees of two sizes—juvenile trees about 2-m tall and aged about 20 years, and mature trees about 5-m tall and aged about 45 years—were selected for sampling at each site. The slope of the land at the sites was 30–40%, with samplings made at 2501, 2596, 2805, 2888, 2990, 3060, 3160, 3260, 3360 and 3450 m. At each site, needles were selected from the upper-third of the canopies of 5–8 trees from each age group.

Carbon isotope composition and leaf nitrogen concentration

Three needle samples (100 needles per sample) were collected at each altitude, and their projected surface area (LA) per sample was determined in the field with an LI-3000A portable area meter (Li-Cor, Lincoln, NE). The measured needles were placed in plastic bags and taken to a laboratory.

The needle samples were oven-dried for 48 h at 80 °C, their dry mass determined and LMA calculated. The samples were finely ground with a mortar and pestle, and each sample was split into two subsamples, of which one was sent to the Lanzhou Institute of Geology, Chinese Academy of Sciences, for determination of $\delta^{13}\text{C}$ and $\%C$. The carbon isotope composition, relative to a PeeDee belemnite (PDB) standard, and $\%C$ was determined by combusting samples in an elemental analyzer coupled to a stable isotope mass spectrometer (MAT252, Finnigan MAT). The overall precision of the ^{13}C analysis was 0.1‰, as tested against a standard with known isotopic composition.

Nitrogen concentration of the needles was determined on the second set of needle subsamples at the Analytical Testing Center, Lanzhou University, China with a CHN analyzer (Vario EL, Elementar, Germany) and leaf N concentration per unit mass (N_m) determined. Leaf N concentration per unit area (N_a) was calculated by multiplying N_m by the LMA.

Stomatal density

Stomatal densities were determined as described by Hultine and Marshall (2000) and Qiang et al. (2003). One hundred and fifty needles from each tree were randomly selected and examined. Stomatal counts were performed on the epidermis of the needles with the aid of a light microscope equipped with a scaled grid. All counts were conducted near the middle of the needle to avoid variations at the base or tip. Stomatal density (number of stomata per unit projected needle surface area) was determined as: $SD = SC/W_n L_n$, where SC is the stomatal count, W_n is the needle width (mm), and L_n is the length of the middle segment of the needle (mm) measured using Motic Images Advanced Software (Version 3.1; www.motic.com).

Because leaves of Qinghai spruce are amphistomatous, SD recorded for all trees was expressed as the mean across both surfaces. We calculated stomatal number per unit length

(SNL) from the sum of the mean abaxial number of stomata per 1 mm row length (McElwain et al. 2002), and per gram of nitrogen (St/N) and per unit leaf mass (St/LM) were calculated by dividing SD by N_a and LMA, respectively.

Statistical analysis

The effects of tree age and elevation on the measured variables were evaluated by analysis of variance. Relationships between elevation and the measured variables were investigated by regression analysis.

Results

Morphological and physiological characteristics of mature and juvenile trees

All measured traits, except for leaf water content (LWC) and leaf carbon concentration (C_m), differed significantly between juvenile and mature trees at almost every altitude (Table 1). Values of $\delta^{13}C$, SD, SNL, LA, LMA and N_a were all significantly higher in mature trees than in juvenile trees (Figures 1–3). Three measured foliar characteristics (N_m , St/LM, and St/N) showed different trends between mature and juvenile trees along the altitudinal gradient. Below 2888 m, N_m was lower in juvenile trees than in mature trees, whereas both St/LM and St/N were higher. From 2888 to 3160 m, there was no distinct difference in N_m between the juvenile and mature trees, whereas St/N was lower in juvenile trees than in mature trees. From 3160 m upward, N_m was lower in juvenile trees than in mature trees, whereas there were no significant differences in either St/N or St/LM between the two age groups (Figures 1C, 2B and 2C). Below 3060 m, LWC was higher in juvenile trees than in mature trees. In contrast, above 3060 m, LWC was lower in juvenile trees than in mature trees (Figure 3C). The C_m of both young and old spruces did not differ significantly at any altitude (Figure 1B).

Relationships between foliar $\delta^{13}C$ and other traits

Foliar $\delta^{13}C$ was correlated with several leaf traits, but these re-

lationships were associated with tree age (Table 2). For example, LMA, N_m and N_a showed no significant difference between the age groups. Generally, the mature trees, located on the steeper slopes, exhibited closer relationships than the juvenile trees (Table 3). In juvenile trees, $\delta^{13}C$ was positively correlated with LMA, N_m , N_a and SNL ($P < 0.001$, $P < 0.01$, $P < 0.001$ and $P < 0.05$, respectively) and negatively correlated with St/N and St/LM ($P < 0.001$ and $P < 0.01$, respectively). In mature trees, $\delta^{13}C$ was positively correlated with LMA, N_m and N_a ($P < 0.001$) and negatively correlated with LWC, LA, SD, St/N, St/LM and SNL ($P < 0.001$). In contrast, C_m was unrelated to $\delta^{13}C$ in either age group.

Trends in foliar ecophysiological traits

All of the measured foliage variables, except LWC and C_m , were significantly affected by tree age and altitude, and these factors interacted (Table 4). The relationships between $\delta^{13}C$, LMA, LA, N_m , N_a , St/N, St/LM and altitude were nonlinear, with a clear transitional zone at around 3100 m (Figures 1–3, Table 5). Values of $\delta^{13}C$, LMA, N_m and N_a of both age groups decreased linearly with altitude at low altitudes (below 3100 m) and increased linearly at high altitudes (above 3100 m). Conversely, LA, St/N and St/LM initially increased and then decreased with altitude (Table 5). The slope of the relationship of $\delta^{13}C$ with altitude was greater for mature trees than for juvenile trees at lower altitude sites between 2501 to 3060 m. Mature trees had about 3 ppm higher $\delta^{13}C$ at the lower altitudes than juvenile trees and about 6 ppm lower $\delta^{13}C$ at the higher altitudes (Table 5). In contrast, there was no linear relationship between LWC or C_m and altitude.

Changes in SD and SNL along the altitudinal gradient suggest a transitional zone at around 3100 m for mature trees (Figures 2A, 2B and 2D, Table 5). From 2501 to 3060 m, SD and SNL increased significantly (by 64.9 and 66.0%, respectively), but from 3060 to 3450 m, SD and SNL decreased by 17.2 and 21.7%, respectively. For the juvenile trees, SD showed no correlation with altitude, whereas SNL increased

Table 1. Significance of the effects of tree age (juvenile versus mature) on structural and physiological leaf traits at each altitudinal site. Probabilities were determined by analysis of variance. Abbreviations: $\delta^{13}C$, ratio of ^{13}C to ^{12}C ; LMA, leaf mass per unit area; LWC, leaf water content; LA, leaf projected area per 100 needles; C_m , leaf carbon concentration per unit leaf mass; N_m , leaf N concentration per unit mass; N_a , leaf N concentration per unit area; SD, stomatal density; St/N, number of stomata per gram of leaf nitrogen; St/LM, number of stomata per unit leaf mass; and SNL, stomatal number per unit length. *P*-values ≥ 0.05 are in bold print.

	2501 m	2596 m	2805 m	2888 m	2990 m	3060 m	3160 m	3260 m	3360 m	3450 m
$\delta^{13}C$	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.736	< 0.001	< 0.001	< 0.001	< 0.001
LMA	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.029	0.003
LWC	0.295	0.374	0.232	0.003	0.365	0.013	0.002	0.519	0.001	0.066
LA	0.005	0.11	0.544	0.462	0.001	< 0.001	< 0.001	< 0.001	0.039	0.004
C_m	0.437	0.806	0.233	0.77	0.812	0.015	< 0.001	0.047	0.6	0.523
N_m	0.016	0.239	0.002	< 0.001	0.078	0.038	0.29	0.031	< 0.001	< 0.001
N_a	< 0.001	< 0.001	< 0.001	< 0.001	0.072	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
SD	0.343	0.048	0.165	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.16	0.038
St/N	0.022	< 0.001	< 0.001	< 0.001	0.001	< 0.001	0.004	0.032	< 0.001	0.063
St/LM	< 0.001	< 0.001	< 0.001	0.047	0.015	0.007	0.001	0.001	0.685	0.489
SNL	< 0.001	0.093	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

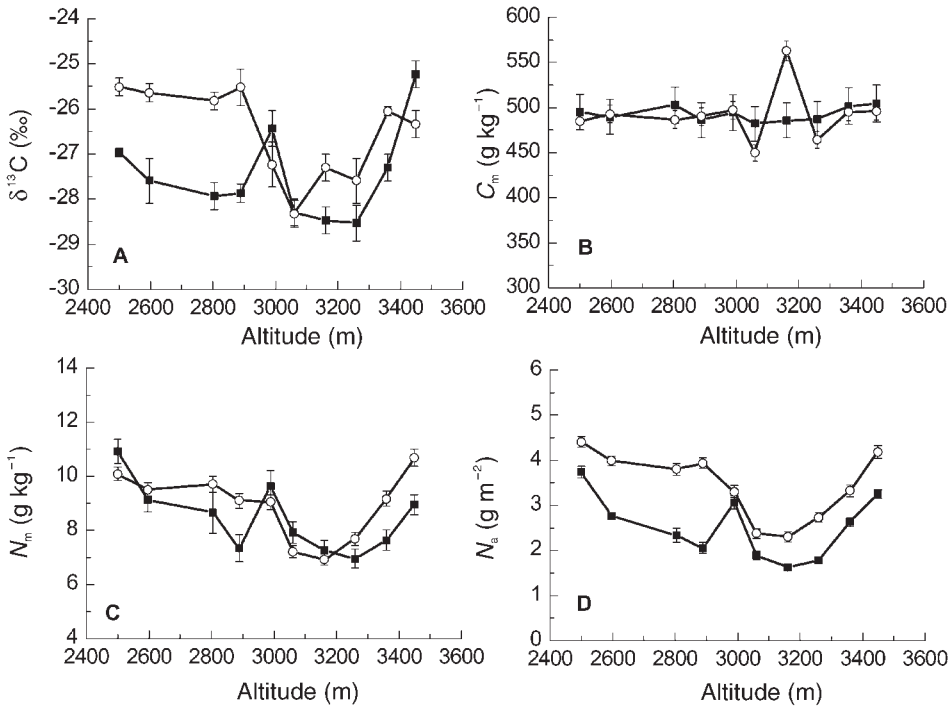


Figure 1. Relationships between altitude and (A) leaf $\delta^{13}\text{C}$, (B) leaf carbon concentration (C_m), and leaf nitrogen concentration on a (C) mass (N_m) and an (D) area (N_a) basis in juvenile (■) and mature trees (○). Values are means \pm standard deviation.

linearly with increasing altitude across all altitude zones (Figure 2, Table 5).

Discussion

Changes in ecophysiological foliar traits of juvenile and mature trees along an altitudinal gradient

Strong correlations have been detected between physiological

traits of trees and altitude for several species, including correlations between $\delta^{13}\text{C}$ and altitude (Körner et al. 1988, 1991, Vitousek et al. 1990, Marshall and Zhang 1994, Sparks and Ehleringer 1997, Hultine and Marshall 2000, Warren et al. 2001). However, these studies concentrated solely on changes in mature trees, neglecting the response and performance of juvenile trees. Our results from a high altitude region showed distinct differences between mature and juvenile trees in some

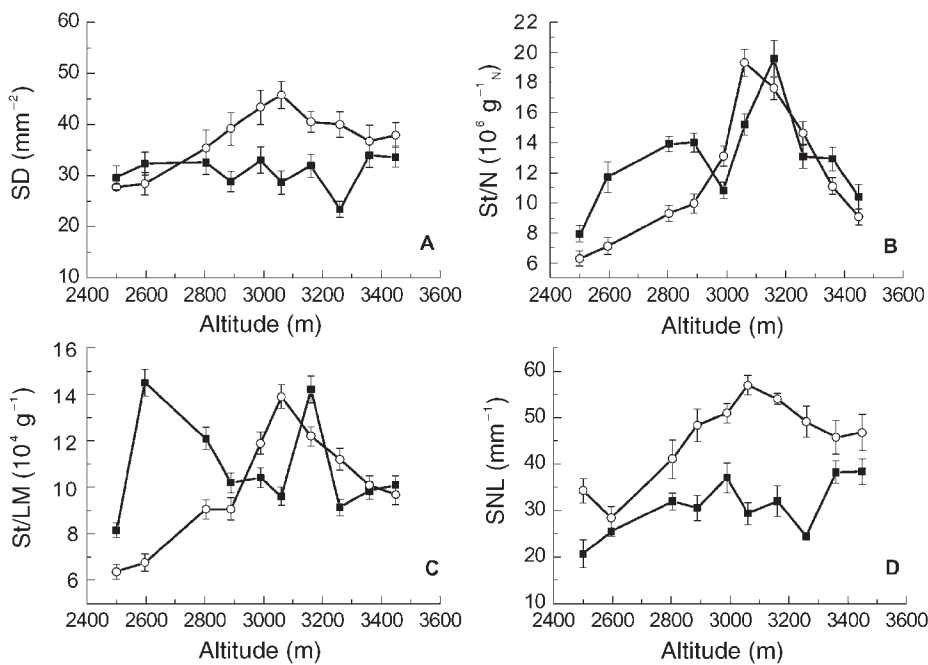


Figure 2. Relationships between altitude and (A) stomatal density (SD), (B) number of stomata per gram of leaf nitrogen (St/N), (C) number of stomata per unit leaf mass (St/LM) and (D) stomatal number per unit length (SNL) in juvenile (■) and mature trees (○). Values are means \pm standard deviation.

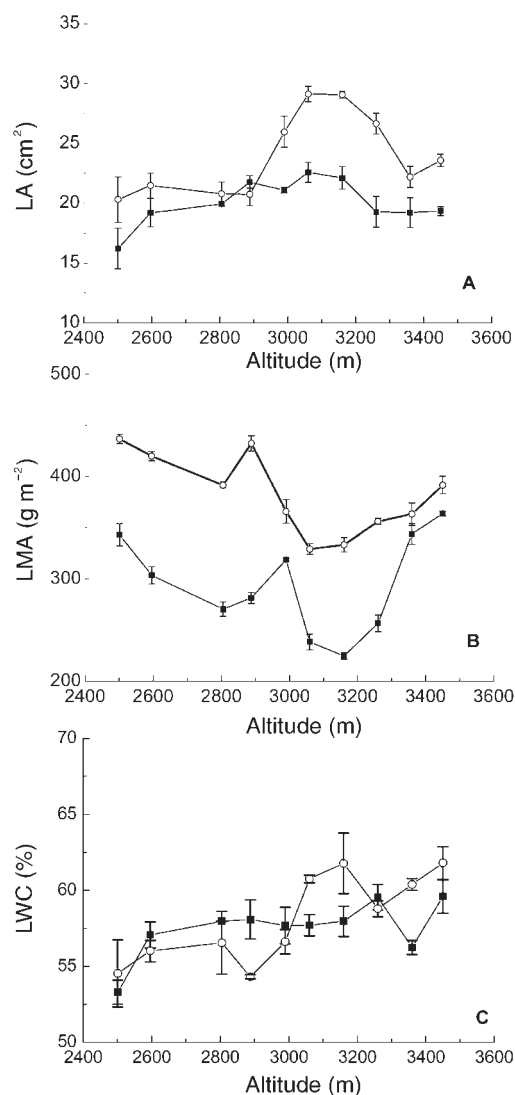


Figure 3. Relationships between altitude and (A) leaf projected area per 100 needles (LA), (B) leaf mass per unit area (LMA), and (C) leaf water content (LWC) in juvenile (■) and mature trees (○). Values are means ± standard deviation.

of the measured variables. First, LMA, $\delta^{13}\text{C}$ and SD were generally, though not consistently, higher in mature trees than in juvenile trees and unaffected by altitude (Table 1). This trend

supports results from previous studies of field-grown conifers and broad-leaved species in low altitude regions (Steele et al. 1989, Yoder et al. 1994, Rijkers et al. 2000, Day et al. 2001, Koch et al. 2004). The leaves of spruces become thicker as trees mature (Richardson et al. 2000). Therefore, the aging of trees may result in a reduction in water limitation of photosynthesis, resulting in increases in $\delta^{13}\text{C}$ and SD. Our results further suggest that this trend is unaffected by altitude (Figures 1A, 2A and 3B, Table 1). Second, LA was typically higher in mature trees than in juvenile trees at both high and low altitudes, except between 2596 and 2888 m (Figure 3A, Table 1). Differences in LA are thought to reflect the transition from the juvenile to the mature stage (Niinemets 2002); however, we found the aging process of Qinghai spruce trees was not consistent at all of the altitudes sampled. Third, N_a was lower in juveniles trees than in mature trees at almost all sites sampled, but the difference in N_m was not consistent between sites, being higher in juvenile trees at two of the ten sites. Rijkers et al. (2000) suggested that N_a may be strongly affected by tree height, but found no correlation between N_m and tree age. Our results confirm the observations of Rijkers et al. (2000) and indicate that there is no correlation between these variables in alpine Qinghai spruce trees growing in high altitude regions. Lastly, St/LM and St/N showed different trends between mature and juvenile trees along the altitudinal gradient because of the different responses of SD, N_m and N_a to altitude. These results collectively indicate that parameters such as LMA, $\delta^{13}\text{C}$, SNL, SD and N_a could be used as stable indicators of tree age and height.

We found that SD of juvenile trees remained relatively unchanged with altitude, whereas SNL increased. In mature trees, both SD and SNL increased with altitude from 2501 to 3060 m and then decreased with altitude up to 3450 m (Table 5). The different altitudinal trends of these variables in juvenile and mature trees are probably associated with the arrangement of stomata in rows or bands parallel to the long axis in needles and the fact that SNL has a stronger relationship with altitude than with SD.

Stomatal parameters may mirror two important physiological processes: absorption of CO_2 in photosynthesis and transpiration of water, both of which are thought to be determined largely by genetic factors (He et al. 1998). However, variations in environmental factors, such as atmospheric CO_2 concentration (Van de Water et al. 1994), water, temperature and sun-

Table 2. Covariance of carbon isotope ratios ($\delta^{13}\text{C}$) against structural and physiological leaf traits. Abbreviations: LMA, leaf mass per unit area; LWC, leaf water content; LA, leaf projected area per 100 needles; C_m , leaf carbon concentration; N_m , Leaf N concentration per unit mass; N_a , leaf N concentration per unit area; SD, stomatal density; St/N, number of stomata per gram of leaf nitrogen; St/LM, number of stomata per leaf mass; and SNL, stomatal number per unit length. *P*-values ≥ 0.05 are in bold print.

	LMA	LWC	LA	C_m	N_m	N_a	SD	St/N	St/LM	SNL
Leaf trait	< 0.001	< 0.001	< 0.001	0.859	< 0.001	< 0.001	0.015	0.082	< 0.001	< 0.001
Ages	0.508	0.012	< 0.001	0.174	0.34	0.265	< 0.001	< 0.001	0.022	0.023
Leaf trait × Ages	0.326	0.009	< 0.001	0.884	0.319	0.32	< 0.001	0.067	0.019	< 0.001
r^2	0.871	0.227	0.7	0.034	0.46	0.781	0.515	0.546	0.495	0.657

light, could also affect changes in SD (Lockheart et al. 1998). For example, SD increases linearly with altitude in a few species (Körner and de Moraes 1979, Körner and Cochrane 1985), whereas in others it shows an initial increase with altitude, but then decreases (Körner et al. 1989, Luo et al. 2006). In other cases SD may remain constant across altitudinal gra-

dients (Woodward 1986). The different patterns of SD with altitude between juvenile and mature trees suggest that, as well as being affected by ecological factors, this variable is related to tree developmental stage.

Neither St/LM nor St/N exhibited consistent increases or decreases across the sampling stations associated with the

Table 3. Regression equations for mature and juvenile $\delta^{13}\text{C}$ values against other structural and physiological leaf traits. Abbreviations: LMA, leaf mass per unit area; LWC, leaf water content; LA, leaf projected area per 100 needles; C_m , leaf carbon concentration; N_m , Leaf N concentration per unit mass; N_a , leaf N concentration per unit area; SD, stomatal density; St/N, number of stomata per gram of leaf nitrogen; St/LM, number of stomata per leaf mass; and SNL, stomatal number per unit length. P -values ≥ 0.05 are in bold print.

Ages	Statistic	LMA	LWC	LA	C_m	N_m	N_a	SD	St/N	St/LM	SNL
Young	Intercept	-32.855	-26.297	-24.958	-34.577	-31.083	-30.328	-29.775	-25.198	-25.128	-29.228
	Slope	0.018	-0.02	-0.125	0.014	0.429	1.138	0.075	-1.7×10^{-7}	-2.2×10^{-5}	0.057
	P	< 0.001	0.84	0.128	0.152	0.002	< 0.001	0.091	< 0.001	0.009	0.039
Mature	Intercept	-34.696	-16.962	-20.958	-28.708	-32.012	-30.455	-22.931	-24.392	-23.238	-23.105
	Slope	0.021	-0.165	-0.232	0.004	0.615	1.141	-0.096	-1.7×10^{-7}	-3.3×10^{-5}	-0.076
	P	< 0.001	0.002	< 0.001	0.482	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Table 4. Covariance of structural and physiological leaf traits against altitude and tree age. Abbreviations: $\delta^{13}\text{C}$, ratio of ^{13}C to ^{12}C ; LMA, leaf mass per unit area; LWC, leaf water content; LA, leaf projected area per 100 needles; C_m , leaf carbon concentration; N_m , Leaf N concentration per unit mass; N_a , leaf N concentration per unit area; SD, stomatal density; St/N, number of stomata per gram of leaf nitrogen; St/LM, number of stomata per unit leaf mass; and SNL, stomatal number per unit length. P -values ≥ 0.05 are in bold print.

	$\delta^{13}\text{C}$	LMA	LWC	LA	C_m	N_m	N_a	SD	St/N	St/LM	SNL
Altitude	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Ages	< 0.001	< 0.001	0.092	< 0.001	0.755	< 0.001	< 0.001	< 0.001	0.01	0.01	< 0.001
Altitude \times Ages	< 0.001	< 0.001	< 0.001	0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
r^2	0.997	0.98	0.796	0.848	0.713	0.932	0.977	0.797	0.934	0.848	0.945

Table 5. Regression equations of structural and physiological leaf traits across the two altitudinal ranges (low altitudes: below 3100 m; and high altitudes: above 3100 m) for juvenile and mature alpine spruce trees (*Picea crassifolia*). Abbreviations: $\delta^{13}\text{C}$, ratio of ^{13}C to ^{12}C ; LMA, leaf mass per unit area; LWC, leaf water content; LA, leaf projected area per 100 needles; C_m , leaf carbon concentration; N_m , Leaf N concentration per unit mass; N_a , leaf N concentration per unit area; SD, stomatal density; St/N, number of stomata per gram of leaf nitrogen; St/LM, number of stomata per unit leaf mass; and SNL, stomatal number per unit length. P -values ≥ 0.05 are in bold print.

Altitude	Statistic	$\delta^{13}\text{C}$	LMA	LWC	LA	C_m	N_m	N_a	SD	St/N	St/LM	SNL
<i>Juvenile</i>												
Low	Intercept	-23.821	611.64	39.451	-6.774	525.85	19.384	8.687	33.033	-1.0×10^7	1.69×10^3	-28.42
	Slope	-0.001	-0.114	0.006	0.01	-0.012	-0.004	-0.002	-0.001	8622.8	37.471	0.021
	P	0.044	0.003	0.002	< 0.001	0.573	0.009	0.002	0.81	< 0.001	0.006	< 0.001
High	Intercept	-64.338	-1428.0	53.738	48.754	267.66	-11.417	-17.059	-22.472	1.0×10^8	5.94×10^5	-78.952
	Slope	0.011	0.522	0.001	-0.009	0.069	0.006	0.006	0.016	-28903	-147.58	0.034
	P	< 0.001	< 0.001	0.77	0.079	0.213	0.005	< 0.001	0.11	< 0.001	< 0.001	0.005
<i>Mature</i>												
Low	Intercept	-14.097	835.79	41.979	-17.116	588.31	19.472	11.491	-57.334	-1.0×10^7	-2.55×10^5	-85.966
	Slope	-0.004	-0.157	0.005	0.014	-0.037	-0.004	-0.003	0.033	19035	124.63	0.046
	P	< 0.001	< 0.001	0.09	0.001	0.111	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
High	Intercept	-42.01	-253.48	55.532	97.647	1094.4	-34.726	-17.923	77.13	1.0×10^8	3.99×10^5	135.92
	Slope	0.005	0.186	0.002	-0.022	-0.178	0.013	0.006	-0.012	-24688	-88.077	-0.026
	P	0.003	< 0.001	0.781	0.001	0.078	< 0.001	< 0.001	0.029	< 0.001	< 0.001	0.001

variations in N_m . Our correlation analysis indicated that, in both juvenile and mature trees, $\delta^{13}\text{C}$ values were significantly correlated with LMA, N_m , N_a , St/N, St/LM and SNL (Table 3), similar to trends reported in previous studies (Hultine and Marshall 2000). Stomatal density, LWC and LA were significantly correlated with $\delta^{13}\text{C}$ values only in mature trees (Table 3). These results imply that correlations between $\delta^{13}\text{C}$ values and other foliar traits strongly depend on tree developmental stage.

Trends in foliar ecophysiological traits of mature and juvenile trees along the altitudinal gradient

For both mature and juvenile trees, variations in $\delta^{13}\text{C}$, N_a , N_m , St/N, St/LM, LA and LMA were not linearly associated with altitude, and at about 3100 m, there was a reversal in their relationships with altitude. The value of $\delta^{13}\text{C}$ is thought to reflect photosynthetic potential and water-use efficiency (Farquhar et al. 1989) and can be correlated with stomatal conductance (cf. Hubick et al. 1986, Hultine and Marshall 2000). Because of the relatively cooler air and lower soil temperatures at higher altitudes—which in turn may reduce fine root activity—water stress can increase and stomatal conductance can decrease with increasing altitude (e.g., Körner 1998, Sveinbjörnsson 2000, Grace et al. 2002). Therefore, $\delta^{13}\text{C}$ is generally thought to increase in high altitude regions (James et al. 1994, Hultine and Marshall 2000). Although Van de Water et al. (2002) reported that $\delta^{13}\text{C}$ measurements in C_3 species show a similar increase with altitude in humid regions, they decrease with altitude in semi-arid regions. These contrasting trends may reflect differences in precipitation at the sampled sites. It is therefore probable that both temperature and water availability affect leaf structure and other physiological traits with increasing altitude in the semi-arid mountain regions. An initial decrease and subsequent increase in $\delta^{13}\text{C}$ values along an altitudinal gradient, similar to that reported here for juvenile and mature trees, has been reported in both mature and juvenile alpine spruces (Qiang et al. 2003, Luo et al. 2006). This variation similarly reflected the different interactions of both temperature and water availability with increasing altitude in the semi-arid Asian highlands. It is probable that in lower altitude regions, as in our study from 2501 to 3100 m, relatively low water stress (because of cooler conditions and higher precipitation), together with increased stomatal conductance as a result of evapotranspiration, and the intercellular concentration of CO_2 , cause $\delta^{13}\text{C}$ to decrease with increasing altitude.

At higher altitude sites (from 3100 to 3450 m) around the tree line, increased water stress, possibly induced by extremely low temperatures, cool air and strong winds (Körner 1998), may enforce stomatal closure, possibly decreasing stomatal conductance and reducing the concentration of intercellular CO_2 . Together these factors may have resulted in the observed increases in $\delta^{13}\text{C}$, LMA and LA. In addition, it has been suggested that leaf N reflects photosynthetic capacity: leaves with high rates of photosynthesis usually have more N per unit area (Evans 1989, Friend and Woodward 1990). However, Stenberg et al. (1995) and Luo et al. (2006) failed to detect this relationship in the spruce species they studied. We

found that N_m and N_a of both mature and juvenile trees were lowest around 3100 m. This altitude may be the optimum zone for growth, because there is a negative correlation between LMA and growth (Walters et al. 1993, Reich et al. 1997) and LMA was observed to be lowest at this elevation.

There were distinct differences in foliar traits between juvenile and mature trees. These differences have important implications for understanding the relationships of leaf morphology with altitude and climate. A few leaf morphological characteristics, e.g., SD, have been used to infer past climatic conditions from fossil or sub-fossil leaves (Beerling and Kelly 1997, 2002, Chen et al. 2001). If plants from different developmental stages differ in morphological plasticity, then the developmental stage of fossil material will be relevant to inferences concerning past climatic conditions.

In this study, relationships between several foliar traits and altitude differed between juvenile and mature trees. These differences suggest that forests of different aged trees may differ in their response to future changes in atmospheric CO_2 concentration and temperature. Both the higher values and variability in $\delta^{13}\text{C}$, LA, SD and SNL in mature trees suggest that forests dominated by older trees could be more affected by climate change than young forests. If true, then artificial planting of seedlings in older forests may increase their potential to adapt to the climatic impacts predicted for high altitude regions.

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